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THE EFFECTS OF PHYSICAL TRAINING UPON FAT CELL SIZE AND NUMBER IN ADIPOSE TISSUE OF THE RAT

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Effects of Physical Training Upon Fat Cell Size and Number in Adipose Tissue of the Rat" submitted by Michael Anthony Booth in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The effects of endurance training upon size and number of epididymal fat cells were investigated in 63 male rats during two successive eight week growth periods, from 7 to 16 weeks and from 16 to 24 weeks of age respectively. The period prior to 16 weeks of age, was considered a developmental period during which time the number of mature fat cells is still subject to change. The second period was a post-development period.

Nine rats, 7 weeks of age, were placed in each of 7 groups: group I was sacrificed initially; groups S and SS were sedentary and groups T and TT were trained on a treadmill until 16 or 24 weeks respectively; group ST was trained only during the post-development period, while group TS was detrained during this period. The caloric intake of all sedentary rats was restricted to that of the training groups. Body weight, pad weight, fatness index (mg epididymal fat/g. body weight) and pad lipid content were determined. Suspensions of fixed, isolated cells, were used for microscopic measurement of cell diameter. Cell number was determined indirectly from the lipid contents of mean cell and whole pad.

Between 7 and 16 weeks of age, cell volume increased 2 - fold in trained rats and 4 - fold in sedentary rats, while cell number showed slightly greater than a 2 - fold increase in both groups. Training, whether early (T), late (ST) or continuous (TT) did not significantly affect cell number, but was effective in maintaining or reducing cell volume to a level approximately half of that found in sedentary rats. Detraining was accompanied by a return of cell size to sedentary levels, so the effects of early training were not sustained.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

STATEMENT OF THE PROBLEM

Introduction

White adipose tissue is by far the largest of the energy depots in the body, but whether it functions efficiently as a metabolizable energy store, or whether it merely contributes to obesity, appears to be a function of the two component parameters: fat cell size and number.

The primary impetus to the cascade of research into the significance of adipose tissue morphology was the discovery by Rodbell (1964a) of an effective procedure for isolating viable fat cells from the quantitatively significant stromo-vascular component. Since that time, two basic, but related, lines of research have been followed: the first concerned with the significance of size to the metabolic potential of the cell, and the second with the chronological development of the tissue and the factors which might induce cell size or number changes during certain critical periods.

Studies of lipid deposition and mobilization in isolated fat cells have indicated definite efficiency advantages in maintaining cell size within functional limits. When

specific doses of hormonal regulators were applied to suspensions containing a given quota of cells, large cells were found capable of metabolizing more lipid than smaller ones, but when the response was determined per unit of stored fat, the metabolic capacity of the smaller cells was considerably greater (Nestel, Austin and Foxman, 1969; Therriault, Hubbard and Mellin, 1969; Greenwood, Johnson and Hirsch, 1970; Bjorntorp and Karlsson, 1970; Goldrick and McLoughlin, 1970; Salans and Dougherty, 1971; Hubbard and Matthew, 1971; Zinder and Shapiro, 1971; Smith, 1971b; Hartman et al., 1971; Bjorntorp and Sjostrom, 1972). Thus, if cell number is fixed, a greater absolute response can be obtained from large cells, but the efficiency of the response is considerably greater in smaller cells.

Goss (1964, 1966, 1967, 1970) has examined the whole question of hypertrophy versus hyperplasia in biological systems, and has hypothesized that the degree of hypertrophy which a cell can undergo, without diminution in functional returns, is largely determined by certain critical ratios in the cell. The ratios considered crucial by Goss, to the control and efficiency of the cell as a unit, are the nucleo/cytoplasmic ratio and the surface area/volume ratio of the cell. In fat tissue, the ratio of protein to lipid volume (Goldrick, 1967a) or, perhaps more precisely, the ratio of active protein to surface area of

the central lipid droplet, would also be important.

The size of fat cells in various adipose depots has been examined in both humans (Hirsch, Knittle and Salans, 1966; Brook, 1970; Bonnet et al., 1970) and in animals (Hirsch and Han, 1969; Hubbard and Matthew, 1971; Johnson and Hirsch, 1972) as a function of age. Cell size has also been studied in obese humans (Hirsch, Knittle and Salans, 1966; Hirsch and Knittle, 1970; Bray, 1970; Englhardt et al., 1971) and in experimental animals in response to cold exposure (Therriault, Hubbard and Mellin, 1969), physical training (Palmer and Tipton, 1972) and various dietary manipulations (Peckham, Entenman and Carroll, 1962; Knittle and Hirsch, 1968; Hirsch and Han, 1969; Hollenberg, Vost and Patten, 1970; Salans, Horton and Sims, 1971; Hubbard and Matthew, 1971; Johnson and Hirsch, 1972; Romsos and Leveille, 1972). All of these stimuli significantly affected adipocyte size, thus indicating that size is a labile property of the cell in both animals and humans. Starvation, cold-exposure and physical training, all of which create an energy requirement, were associated with reduction in fat cell size.

Results in terms of cell number have been somewhat less definite. The studies of Enesco and Leblond (1962) have indicated that most organs and tissues of the rat

acquire their full adult complement very early in life. Results with adipose tissue have shown the same pattern (Knittle and Hirsch, 1968; Hirsch and Han, 1969). Studies with humans (Bray, 1970; Bjorntorp and Sjostrom, 1971; Englhardt et al., 1971) and with animals (Johnson and Hirsch, 1972) have shown that certain extreme states of obesity in the adult are associated with a much expanded fat cell complement. However, in normal animals, the final adult quota of fat cells has proved remarkably resistant to experimental adjustment. The only functional stimulus which has been found effective in modifying adult fat cell number has been undernutrition in rats less than three weeks of age (ie, pre-weaning animals) (Knittle and Hirsch, 1968). The effects of other stimuli have not been investigated in rats at this young age.

Rationale Behind the Study

From the foregoing discussion, it would appear that a large number of small cells would be advantageous in an animal which was required to store and mobilize, rapidly and frequently, large amounts of lipid. Such would be the case in rats undergoing daily endurance training, since lipids provide the major source of energy for working muscles (Cobb and Johnson, 1963; Havel et al., 1964; Issekutz and Paul, 1966). Palmer and Tipton (1972) have found a limiting effect of training upon cell size in rats, but no studies

on cell number have yet been reported.

In a static organ or tissue, such as adipose tissue, a cellular commitment is irreversible; cells, once formed, remain for the life-span of the organism (Goss, 1970). Consequently, an animal might be extremely reluctant to compensate for a functional overload, which may prove transient, by a local change in the proliferative response. If additional fat cells were formed in response to training during an early developmental stage, and the lipid energy demand then fell, as in a subsequent detraining period, the large cell complement would provide the potential for development of more extreme states of obesity.

The use of experimental animals. The use of experimental animals, in the study of adipose tissue morphology, has been necessitated by the tremendous obstacles which restrict the use of human subjects. Human studies have obvious advantages since the generalizations which can be made, from a small rodent with a high metabolic rate and a short life-span, to man, are obviously limited. However, the factors which affect the development of adipose tissue have been more fully researched, and are better understood, in rats than in man. Moreover, the critical periods in growth are much more clearly defined, so experimental treatments can be more meaningfully applied and evaluated.

Purpose of the Study

The purpose of the study was to determine the effective capacity of daily endurance training to modify the size and number of fat cells, in the epididymal pad, at various stages in the growth of the rat. Endurance training programs were conducted both before and after the developmental period. The effects of a continuous training program, and of post-developmental detraining, were also examined.

Limitations and Delimitations of the Study

1. The study was confined to the epididymal fat pads of male Wistar rats between the ages of seven and 24 weeks.

2. Only one intensity of endurance training was used, and some trauma could have resulted from the presence of the electrical shocking device on the treadmill.

3. The methods used for cell counting were indirect and consequently subject to some error.

4. Only crude estimates of food intake were obtained.

CHAPTER II

REVIEW OF THE LITERATURE

Significance of Adipose Tissue as an Organ

Forty years ago, adipose tissue was thought to be a connective tissue filled, by chance, with droplets of fat (Wertheimer, 1965). The tissue was considered devoid of nerves and sparsely supplied with blood vessels. The ascribed functions were the completely passive ones of insulation and padding.

Today, adipose tissue is considered, by many, as an organ in its own right, embryologically differentiated (Wasserman, 1965). In homoiotherms, it has an active thermogenic function and Vague and Fenasse (1965) have implied that it may be assuming some of the duties of the liver. As an active center for energy homeostasis, it assumes central importance in some of the present-day problem diseases such as obesity, diabetes and atherosclerosis (Gordon, 1970; Bjorntorp and Ostman, 1971). The rich innervation and blood supply enable precise neural-hormonal control of lipid deposition, mobilization and turnover, and permit response to such stimuli as caloric

intake, diet, cold and cold acclimation, mental and emotional strain, and exercise (Wertheimer, 1965). In prolonged exercise, lipids form the major source of energy for the working muscles (Cobb and Johnson, 1963; Havel et al., 1964). In relation to its protein content, adipose tissue is metabolically more active than liver (Hausberger et al., 1954) and has a greater vascularity than muscle (Gersh and Still, 1945).

Genesis, Histology and Compartmentation in Adipose Tissue

Several different views on the origin of adipose tissue are still held. Napolitano (1965), on the basis of electron microscope studies, adheres to one of the early views of Fleming that the primitive white fat cell is "indistinguishable from the typical fibroblast." Wasserman (1965) and Simon (1965), however, using the light and electron microscope respectively, have concluded in favour of an origin from perivascular reticulo-endothelial cells; Simon favours a reticular and Wasserman an endothelial, origin. Both authors consider the penetration and ramification of blood capillaries within the primitive mesenchymatous lobules to be an essential precondition for the deposition of fat in the cells. This anlage of fat lobules seems to be the same in all vertebrates studied, including the rat and man (Wasserman, 1965).

Simon (1965) has described the primitive adipogenic

reticular cell as having an elongated nucleus, and scant basophilic cytoplasm. When lipids begin to accumulate, they appear in the cytoplasm as numerous small inclusions (liposomes). These liposomes soon fuse and form large globules around the nucleus, which still occupies a central location in the cell. With further maturation, the lipid droplets coalesce to form a large central lipid cake, forcing the nucleus to the periphery and giving the typical "signet-ring" appearance to the cell (Napolitano, 1965). The cytoplasm which surrounds the nucleus and forms a thin rim around the spherical fat-cake, contains mitochondria, microsomal material, glycogen and liposomes (Simon, 1965; Angel, 1970). Enclosing the cell contents is a plasma membrane and a basement membrane, surrounded by a network of collagen fibers (Napolitano, 1965). The central fat cake is believed to be a relatively inert storage compartment, while the cytoplasmic compartment, containing the organelles and liposomes, is metabolically very active (Hirsch et al., 1960; Jeanrenaud, 1961; Wertheimer, 1965; Angel, 1970).

Distribution and Function of Fat in the Body: Age, Sex and Species Differences

The intermittent eating habit of most animals has posed the problem of temporarily storing much of the energy ingested as food. In the steady-state, about 30 per cent

of all the carbohydrate ingested is temporarily converted to fat (Wertheimer, 1965), which not only occupies less volume but also weighs much less, per calorie of stored chemical energy, than does carbohydrate or protein (Weiss-Fogh, 1967). As the caloric input increases, this proportion may be greater than 30 per cent.

The white adipose tissue of most mammals is either subcutaneous or "deep." The deep fat is primarily perivascular, peritoneal or intermuscular and exists as distinct pads, while the subcutaneous fat is more fibrous and lobular, and lies between the skin and the superficial fascia. Despite the fact that considerable overlap occurs in the functions of the various depots, some adaptation to specific functions is reflected in structural and compositional differences (Di Girolamo and Mendlinger, 1971; Johnson and Hirsch, 1972), as it is doubtful whether any one fat type or site could be considered truly representative of body fat in general.

Subcutaneous fat has appeared in evolution simultaneously with the appearance of homoiothermy (Wertheimer, 1965) and is most abundant in those mammals which lack fur or hair or which have become acclimated to the cold (Vague and Fenasse, 1965). In man and in the rat, subcutaneous fat comprises more than half of the total adipose mass (cited by Keys and Brozek, 1953), but the fraction may not be constant.

In humans, the extent and location of the subcutaneous deposition varies widely with sex, age and activity (Keys and Brozek, 1953; Vague and Fenasse, 1965). A passive insulatory role has been accredited to the tissue, but evidence also exists for an active calorigenic function. In this respect, an electric blanket analogy has been applied to the layer of subcutaneous fat.

The various adipose types and sites may not respond equally to applied metabolic stimuli. In general, internal depots appear to be more active metabolically than subcutaneous fat, which in turn is more active than fat with a primary supportive function (Wertheimer, 1965; Goldrick, 1967b). Preferential use of hump fat by camels on long trips, and of deep rather than subcutaneous fat in hibernating animals, as well as resistance to mobilization of the ethnic steatopygias of African Bushmen and Hottentots, all exemplify the capacity for differential metabolic activity in the various fat depots.

The Epididymal Fat Pad of the Rat

Since the rat is a small animal with a life-span less than two years and a high metabolic rate in relation to total fat stores (Bjorntorp and Ostman, 1971), obvious questions could be raised regarding the degree to which rat tissue is representative of fat tissue in man. However,

the use of larger animals, or of human subjects, introduces severe legal, practical and experimental restrictions. A biopsy technique (Hirsch et al., 1960) can be used to obtain human samples under experimental conditions, but the validity of generalizing from one small, subcutaneous biopsy specimen to the total adipose mass, is questionable.

The metabolic responses of rat epididymal fat are, in some respects, both qualitatively and quantitatively different from those of human tissues (Bjorntorp and Ostman, 1971), but the morphology and growth patterns have borne a much closer resemblance. Consequently, rat tissues have been used in numerous studies of adipose tissue morphology. The epididymal pads have proved the most popular because they are metabolically active and are easily located and removed in toto from the body.

Jeanrenaud (1961) has cited evidence that the left epididymal pad is metabolically more active than the right, and consequently, the vast majority of researchers have shown a preference for the use of the left pad in metabolic studies. Hirsch and Han (1969), however, have reported no differences between the right and left pads with respect to pad weight, cell size and cell number, but the comparisons were made on group means. Studies in this laboratory have indicated that, for an individual animal, the larger pad may

be as much as 25 per cent heavier than the smaller one. Differences in either cell size or cell number presumably must account for most of the weight difference. Consequently, it would seem preferable, in morphological studies, to choose either the larger or the smaller pad, rather than the left or the right, thus reducing the between animal variation.

Classification of Tissues: Expanding, static, renewing

All embryonic tissues and organs are constantly expanding, because both daughter cells, from a single cell division, continue to divide (Enesco and Leblond, 1962). As development progresses, however, the daughter cells may either retain their germinal properties, differentiate but remain able to divide, or differentiate and lose mitotic capacity (Lehmiller, 1971). These alternative proliferative responses have prompted Messier and Leblond (1960) to classify adult tissues as either static, expanding or renewing. The characteristics of each type have since been extensively detailed by Goss (1964, 1967, 1970):

(i) Static tissues are those in which all cells differentiate and in so doing lose the capacity to divide beyond late embryonic or early childhood stages. The majority of cells live as long as the organism, but if the life-span is extended, as in humans, then those cells which

die prematurely do so without replacement. Classically, this group has been represented by nerve, skeletal and cardiac muscle fibers, but fat cells probably also belong (Goss, 1966; Hirsch and Han, 1969; Hollenberg, Vost and Patten, 1970). The inability of differentiated cells to divide appears to be correlated with the storage in the cytoplasm of specialized end-products, such as the myofilaments of muscle, the keratin of the epidermis, and the fat cake of adipocytes (Goss, 1960).

(ii) Expanding tissues exhibit birth but not death of cells. All cells differentiate, as in static tissues, but retain their mitotic capabilities until an advanced age. Though these cells can divide, they do not unless appropriately stimulated by tissue loss or maintained physiological overload. Most of these tissues have considerable capacity for metabolic adaptation, so morphological adjustments are seldom necessary. Liver, kidney and most endocrine and exocrine glands belong in this category.

(iii) Renewing tissues exist where wear-and-tear, tissue loss or infection may be a problem (eg. blood cells, lymph cells, epidermis, alimentary mucosa). These cells differentiate rapidly and old cells are continually being replaced by new cells from germinative growth zones.

Mature adipose tissue has been classed by Goss (1966,

1970) as a non-mitotic tissue, and his decision appears justified in the light of recent research (Hirsch and Han, 1969; Hollenberg, Vost and Patten, 1970). If adipose tissue truly belongs in this category, then there is no capacity for cellular turnover. All turnover in response to functional demands must be at the molecular or organelle level, and all tissue growth in adults must be accomplished by cellular hypertrophy. The potential for growth and adaptation in this situation would be severely limited (Figure 2.1).

Some degree of overlap in tissue categories has been recognized, however (Figure 2.2). With regard to adipose tissue growth and adaptation, the question would seem to be whether adipose tissue can retain, into adult life, any capacity to expand. This could occur in any of three ways; either the mature cells could divide, dedifferentiation could occur, or germinative cells could persist in a dormant state.

With regard to the first alternative, mature fat cells have been observed microscopically in a polyploid or binucleate state (Mohr and Beneke, 1968), but never in the process of cell division. How such division could occur in the fully differentiated, gorged fat cell would, indeed, be hard to visualize. Dedifferentiation is likewise, seldom observed in static tissues, except under very unusual circumstances (Goss, 1967). The most likely prospect for expansion of

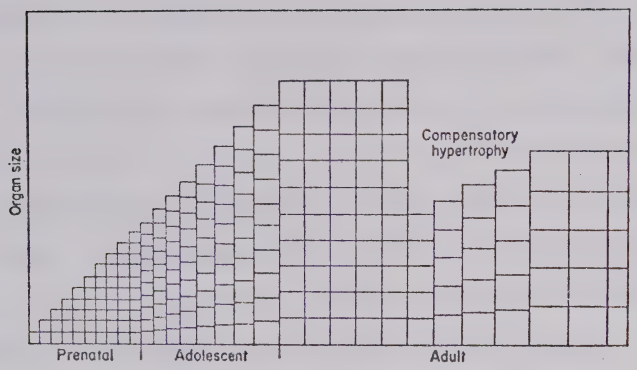

FIGURE 2.1 GROWTH IN AN ORGAN OF LIMITED GROWTH POTENTIAL
Each square indicates *n* functional units, which can proliferate only during early developmental periods. Subsequent growth occurs solely by hypertrophy, whether it be normal maturation to adult dimensions or compensatory growth after partial ablation (from Goss, 1967).

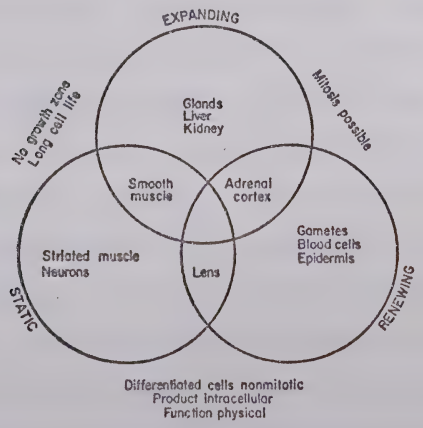

FIGURE 2.2 CLASSIFICATION OF TISSUES ACCORDING TO MODES OF GROWTH AND TYPES OF DIFFERENTIATION
Exceptions to the general rule are listed in overlapping areas (from Goss, 1967).

mature fat tissue would be the existence of dormant primordial cells, but these, if they existed, would be extremely difficult to detect and identify. In skeletal muscle, the existence of such "satellite cells" has been proposed. Nevertheless, if such primordial cells do exist in adipose tissue, they would be sparsely located and the capacity for expansion would be so limited as to be largely of academic interest only. A more meaningful question is the morphological change, if any, which can be induced in early life. The values of hypertrophy versus hyperplasia relative to morphological change are also of interest.

Hypertrophy versus Hyperplasia

When a functional unit, such as a cell, is subjected to an overload, it first tends to adapt by means of rapid physiological adjustments, but if the stimulus persists, morphological compensations must be made (Goss, 1964). From an analysis of a wide variety of biological systems, Goss (1966, 1967) has concluded that the first priority in morphological adjustment is for growth in size, but since excessive enlargement ultimately reduces physiological efficiency, the organism attempts to multiply its functional units whenever possible. The reduced efficiency with increased cell size is considered by Goss to be the result of a decline in the surface area/volume ratio and the nucleo/cytoplasmic ratio. The most obvious way to restore

these ratios would be by cell division.

The reluctance of animals and species to make morphological adaptations by means of cell size increases has been clearly indicated in ontogenetic and phylogenetic studies on various types of tissues (Goss, 1964). In these studies, small species have been compared with larger species or else the same species has been compared at progressive stages of growth and development. Mitotically competent cells (such as hepatic, parenchymal or adrenal cortex) are approximately the same size, regardless of body size or species size, thus indicating an obvious preference to increase cell number, and to maintain cell size constant, whenever cell proliferation is possible (Goss, 1967). However, in static tissues, such as nerve and skeletal muscle, the fibers are both larger and more numerous the bigger the animal and the bigger the species (Goss, 1967)*. These tissues appear to resort to cellular hypertrophy because of an inadequacy for hyperplastic compensation. This inability of static tissues to increase cell number is probably a biological safeguard to prevent a permanent commitment to too many cells. If extra cells are formed, they would pro-

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*Goss (1964) has drawn attention to the nephron, the functional unit of the kidney, which is also not able to increase in numbers. Sperber (1944) has found the phylogenetic relation between nephron tubuli size and species size to be logarithmic; ie, tubule size is approaching an asymptotic maximal value in the larger species, probably due to functional efficiency considerations.

vide an additional margin of safety against future overload, but the cells would then have to be permanently maintained by the body.

Thus, an increase in cell number would seem to be the method of choice in morphological adaptation, but if proliferative capacity is limited, then the tissues must resort to cellular hypertrophy. The limits of compensation are then set by efficiency considerations involving the surface-area/volume ratio and nuclec/cytoplasmic ratio of the cell. When functional demands exceed these limits, serious pathological consequences may ensue (Goss, 1967).

Regulation of Cellular Proliferation

There are two predominant theories on the nature of growth regulating factors. The first is the functional demand theory expounded by Goss (1964) and supported by Swann (1958) and by Lehmiller (1971). These authors claim that the products of physiological activity or functional sufficiency are the most obvious candidates for the position of growth regulators (Goss, 1964). Humoral communication with physiologically related tissues is thought to be achieved, not by a specific chemical agent, but by a more general change in the internal environment, perhaps involving numerous chemical and physical factors (Lehmiller, 1971).

The second major theory has been detailed by Weiss and Kavanau (1957) and by Bullough (1962) and is favoured by Cameron (1971) as the most comprehensive model to explain growth and proliferation control (Figure 2.3). This theory postulates the existence of tissue specific inhibitors which are being constantly produced by the differentiated cells and released into the circulation. These substances, called chalones by Bullough (1962) and anti-templates by Weiss and Kavanau (1957) are thought to operate in a negative-feedback fashion to specifically inhibit mitosis, either directly or indirectly, in all tissues homologous to that which produced them. The degree of inhibition is thought to be proportional to the plasma concentration of chalone, which in turn, is proportional to the number of differentiated cells present. Hence, proliferation is regulated by the tissue mass and the cell number is self-limiting. In support of this theory, Bullough and Laurence (1964a) and Hondius-Boldingh and Laurence (1968) have reported success in extracting from the epidermis an anti-mitotic chemical messenger which they have called the epidermal chalone. This substance was found to be tissue specific but not species specific. Turkington (1971) reports that substances with similar characteristics have been detected in liver, kidney, granulocytes and lens.

Goss (1964) has questioned the theory on the

grounds that it permits insufficient capacity for functional adaptation, but several recent papers by Bullough (1965) and Bullough and Laurence (1964b, 1968) have emphasized the interaction of hormones with chalones in regulating mitotic activity. The inhibitory action of epidermal chalone was potentiated by physiological concentrations of epinephrine (Bullough, 1965; Bullough and Laurence, 1964b) and hydrocortisone prolonged the inhibitory effect of the epinephrine-chalone complex (Bullough and Laurence, 1968). The authors have suggested that the diurnal variations which occur in cell proliferation (ie., lowest during the working hours and maximal during rest and sleep) can be explained by the effects of body activity upon the circulatory levels of these hormones. This linkage with metabolically active hormones would seem to make the chalone theory now much more compatible with the concept of response to functional load. The two theories are otherwise similar in that they both emphasize auto-inhibition by systemically disseminated chemical messengers (Goss, 1964).

Other studies with various tissues have confirmed the significance of hormones in regulating cell differentiation and proliferation and have emphasized especially the relation to stress and physical activity. Halberg (1960, 1963) has detected, in a number of tissues, an association between the inhibition of mitotic activity and

the diurnal variation of ACTH and glucocorticoid hormone secretion. Similarly, Rasanen (1963) has found that a single injection of ACTH, or a period of physical stress, produces, within five hours, a decrease in the mitotic index of the rat gastric mucosa. With continued ACTH stimulation, the mitotic index gradually returned to normal and subsequently exceeded the control value. Lahtiharju and Teir (1964) and Lahtiharju, Rasanen and Teir (1964) have shown that similar changes can be produced in liver and other organs by injections of glucocorticoids or heparin. Rasanen (1967) has proposed that, in the gastric mucosa at least, the glucocorticoid hormones may act to degranulate the large numbers of mast cells which are present and that the heparin so released may cause the hyperplastic inhibition observed. Cell proliferation in adipose tissue has not been tested in response to these hormones.

In contrast to the inhibition observed in response to the catabolic hormones, insulin was found to exert a potent effect upon hepatocyte proliferation in alloxan diabetic rats (Younger, King and Steiner, 1966). In adipose tissue similar results were obtained (Hollenberg, Vost and Patten, 1970). Alloxan, when administered to rats, decreased the rate of cell proliferation in both the stromal and fat cell fractions. Administration of both insulin and growth hormone increased DNA synthesis, but in the stromal elements only.

Finally, Perris (1971) has reviewed an extensive series of experiments which strongly implicate calcium ions and cyclic - AMP in the stimulation of cell proliferation. These experiments were conducted on the lymphopoietic and erythropoietic systems of rats in collaboration with Whitfield, MacManus, and coworkers (cited by Perris, 1971). When calcium levels in the plasma or medium, or cyclic-AMP levels in the cells were altered by a variety of different techniques, a stimulating effect upon mitogenic activity was always observed. Also, as the daily growth rate of the rat increased and then subsequently declined, closely correlated changes were observed between tissue mitotic activity and plasma concentrations of ionized calcium (Perris, Whitfield and Tolg, 1968). A scheme (Figure 2.4) has been proposed by Perris
membrane (with or without a sensitizing agent such as the detergents, bradykinin, parathormone, growth hormone, prolactin and the neurohormones) to raise the intracellular concentration of cyclic - AMP and disrupt the DNA - histone complex.

Although the regulating mechanisms discussed have only been studied extensively in the rapidly renewing tissues of the body, they may have equal significance in the more static tissues during the early periods of growth when these tissues are still expanding.

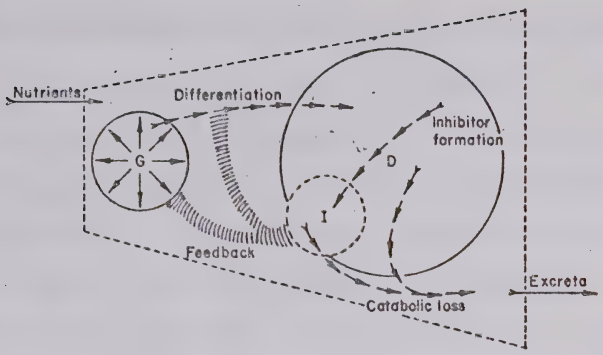

FIGURE 2.3 THE WEISS-KAVANAU MODEL OF GROWTH CONTROL
G means generative compartment, D means differentiating compartment, I means inhibiting principle. Cells from the generative compartment are transferred to the differentiated compartment and from there lost by catabolism. The differentiated compartment produces an inhibitor substance which diffuses back and probably regulates both the cell proliferative activity of the generative compartment as well as the rate of cell differentiation. This model can be used to explain the experimental findings concerning the regulation of cell proliferation in the cell populations of the mammalian body (from Weiss and Kavanau, 1957).

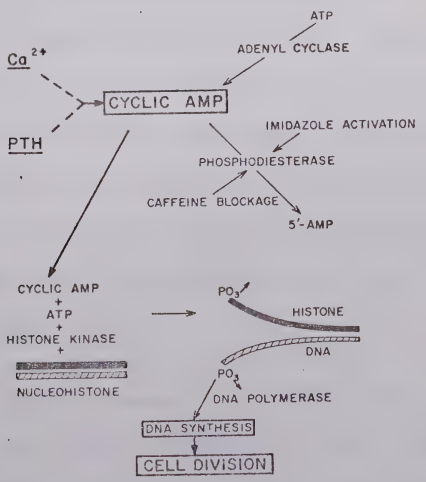

FIGURE 2.4 PROPOSED REACTION SCHEME FOR CALCIUM-, PTH-, AND CYCLIC AMP-INDUCED MITOGENESIS
(from Perris, 1971)

Methodological Procedures for Counting and Sizing Fat Cells

The lipid content and metabolic capacity of a fat pad of given weight may vary considerably as a result of variations in the size and number of its component fat cells and of the proportions of stromal elements (Goldrick, 1967a; Hubbard and Matthew, 1971; Di Girolamo and Mendlinger, 1971). The stromo-vascular component of the tissue is not a constant factor of the weight of the tissue (Hollenberg, Vost and Patten, 1970; Hubbard and Matthew, 1971; Di Girolamo and Mendlinger, 1971) and the lipid content of the individual fat cells is not a constant portion of fat cell weight (Goldrick, 1967a; Englhardt et al., 1971; Salans and Dougherty, 1971; Bjorntorp and Sjostrom, 1972). Factors such as age (Goldrick, 1967a), diet (Hubbard and Matthew, 1971) and exercise (Grollman and Costello, 1971) can all affect the per cent lipid content, either by modifying the size of the fat cell or its lipid/protein ratio, or by differential effects upon the development of stroma and adipocytes. Consequently, researchers have attempted to better classify the state of adiposity by measuring the fat cell size and number. Methodology has been the primary obstacle to progress.

In recent years, extensive methodological advances have been made in the study of adipose tissue morphology

(Rodbell, 1964a; Goldrick, 1967a; Wasserman, Elias and Tyler, 1967; Martinsson, 1968; Hirsch and Gallian, 1968; Bjorntorp and Karlsson, 1970; Hollenberg, Vost and Patten, 1970; Sjostrom, Bjorntorp and Vrana, 1971; Di Girolamo, Mendlinger and Fertig, 1971; Smith, 1971a). The techniques used in these studies have ranged from microscopic analyses to histological sections and fresh tissue pieces to electronic cell counting, DNA labelling with isotopic thymidine and the preparation of fat cell cultures. Most researchers have resorted to the use of isolated fat cell suspensions, prepared either by the collagenase digestion technique of Rodbell (1964a) or by the disruption of fat tissue with osmium tetroxide fixative (Hirsch and Gallian, 1968). In the majority of cases, cell size has been determined either directly by microscopic visualization or semi-automatically from photomicrographs, but the Coulter counter has been used to plot size distributions electronically. All counts have been popularly obtained by one of two methods: by direct electronic counting of isolated osmium fixed cells or indirectly from pad lipid content and cell size. Other methods of counting have involved DNA determinations or the use of microscopic counting chambers. Each method of counting and sizing has certain limitations and advantages.

The ultimate aim of the researcher is to obtain a valid estimate of cell size and/or number in the simplest

and most economical way possible. Thus, the four factors of primary concern are representative sampling, valid measurement, facilitation of methodological and technical difficulties and economy of time and expense.

The initial problem of representative sampling has been best overcome by the use of the whole fat pad (or of numerous, small, randomly-drawn samples of fat tissue) for the preparation of isolated fat cell suspensions. The use of osmium for fixation and disruption followed by electronic counting and sizing (Hirsch and Gallian, 1968; Sjostrom, Bjorntorp and Vrana, 1971) has obvious advantages in terms of time economy and results in minimal cell loss and breakage. However, technical problems and expense may be extensive. If collagenase digests (Rodbell, 1964a) are used as the alternative, then cell rupture and size change should be minimized by the use of passive flotation instead of centrifugation (Martinsson, 1968), and by chilling and stabilizing the cells in a mild fixative such as TCA-GTA or formaldehyde (Hirsch and Gallian, 1968; Sjostrom, Bjorntorp and Vrana, 1971). The cells could then be sized under a microscope directly or photomicrographs could be taken for semi-automatic sizing at a later date, for example, with a Zeiss analyzer (Goldrick, 1967a; Hirsch and Gallian, 1968). Since cell loss and rupture may be extensive by the collagenase procedure, cell counts in this instance are best obtained by an indirect method, requiring analyses of

pad lipid content and estimation of mean cell lipid content from cell diameter (Hirsch and Gallian, 1968; Di Girolamo, Mendlinger and Fertig, 1971).

Measurement of Proliferative Capacity and Primordial Cells

The commencement of lipid accumulation in adipose cells is considered to predispose the cell to a non-mitotic state (Goss, 1970). Also, dedifferentiation of any non-mitotic tissue is a very exceptional occurrence (Goss, 1967). Consequently, any capacity which adipose tissue has, to expand its fat cell complement, must reside in the presence or absence of undifferentiated cells in the tissue. These primitive cells could presumably be in one of two states: a germinative state, in which mitotic capacity is retained, but the cell remains dormant until stimulated to proliferate; or a primordial state in which mitotic capacity is not retained, but the cell remains dormant until stimulated to differentiate. The most probable situation would be one in which the majority of cells would have germinative properties in the very early years and primordial properties thereafter. At present, however, the primordial cell must remain a hypothetical entity because adequate methods are not available for their visualization in histological sections or measurement in fat cell fractions.

Wasserman (1965) has reported that cells believed

to be histocytes or fibroblasts have frequently been observed among fat cells in histological preparations and "it is conceivable that they represent a stock from which new fat cells may be supplied." In this sense, they would resemble the "satellite cells" which have been described for muscle. The uncertainty which exists, however (Wasserman, 1965; Simon, 1965; Napolitano, 1965), regarding the exact nature of adipocyte precursors has made the positive identification of such cells in histological preparations impossible up to this time. Cells cannot be clearly distinguished as pre-adipocytes until they begin to accumulate lipid.

Similarly, in fat cell fractions, isolated by collagenase, primordial cells will not be counted or sized because they contain insufficient lipid to float with the mature fat cells (Salans and Dougherty, 1971). Hollenberg, Vost and Patten (1970) have estimated that five to seven days are required, after DNA synthesis, for cells to accumulate sufficient lipid for maximal recovery by flotation. Consequently, if labelled thymidine was administered, division and maturation of germinative cells could be detected by the appearance of label in the fat cell fraction, but if primordial cells differentiated in this same period, they would go undetected.

Finally, consideration must be given to the perivas-

cular origin of adipose tissue (Wasserman, 1965; Simon, 1965). In view of this proposed origin, the possibility exists that any stimulus which leads to increased capillarization of adipose tissue could produce an increased stock of fat cells. In this case, the expansion of adipose cell number would occur secondarily to the formation of new blood vessels.

Metabolism of Adipose Tissue in Relation to Cell Size

When intact fat cells are liberated from adipose tissue by collagenase digestion, they retain their intrinsic capacity to synthesize tryglyceride, to oxidize glucose and to respond to lipogenic and lipolytic hormones (Rodbell, 1964a). These properties of the isolated cell suspension have inspired numerous studies on the relation between cell size and metabolism. If metabolic response is independent of cell size, then the number of cells present will determine the metabolic response of the tissue. If, however, a size dependency does exist, then the various size-dependent cell components (and the ratios that exist between them) must be examined as an explanation for the varying metabolic response. Cell lipid content, cytoplasmic mass, membrane surface area and enzyme concentrations have all been studied in this regard.

When cells of different sizes were compared for their ability to synthesize lipid from glucose (Zinder, Arad and

Shapiro, 1967; Nestel, Austin and Foxman, 1969; Goldrick and McLoughlin, 1970; Bjorntorp and Karlsson, 1970; Smith, 1971b; Bjorntorp and Sjostrom, 1972), to esterify labelled fatty acids in the presence of glucose (Zinder, Arad and Shapiro, 1967; Nestel, Austin and Foxman, 1969; Bjorntorp and Sjostrom, 1972) or to respond to insulin (Nestel, Austin and Foxman, 1969; Greenwood, Johnson and Hirsch, 1970; Salans and Dougherty, 1971; Salans, Horton and Sims, 1971; Bjorntorp and Sjostrom, 1971; Smith, 1971b), obvious differences were observed. The larger cells, in each case, showed a greater absolute response per cell, but a lesser capacity per unit weight of lipid. This has been found to be true, whether large and small cells were obtained from animals of different age (Zinder, Arad and Shapiro, 1967; Nestel, Austin and Foxman, 1969; Salans and Dougherty, 1971), or from animals or patients in various states of obesity (Salans, Knittle and Hirsch, 1968; Greenwood, Johnson and Hirsch, 1970; Bjorntorp and Sjostrom, 1971). The same relationship was found when large and small cells were obtained from the same patients before and after weight loss (Bjorntorp and Sjostrom, 1971; Salans, Horton and Sims, 1971). Finally, confirmation has come from studies in which cells, obtained from the same pads of the same subjects at the same time, were separated into fractions of different mean size and compared in terms of metabolic capacity (Bjorntorp and Karlsson, 1970; Smith, 1971b; Bjorntorp and Sjostrom, 1972).

Exceptions to the general trend of results have been observed (Salans and Dougherty, 1971) but such studies are few.

When similar comparisons were made in response to lypolytic hormones, the pattern of results was similar (Therriault, Hubbard and Mellin, 1969; Hubbard and Matthew, 1971; Zinder and Shapiro, 1971; Hartman et al., 1971; Bjorntorp and Sjostrom, 1972). The larger cells were found to be less sensitive to lipolytic hormones (ACTH, norepinephrine and epinephrine), although absolute release of free fatty acid per cell was greater.

These findings imply that, for a given fat cell complement, larger cells can both deposit and mobilize stored fat more quickly. In relation to the total amount of lipid stored, however, the response of the larger cells is considerably less (ie, there is less sensitivity to a prescribed dose of hormone).

These studies, then, have revealed a definite dependency of the metabolic response upon fat cell size. It is thus necessary to determine the various size-dependent components of the cell and to determine which of these could be responsible for the metabolic variations observed.

Enlargement of the fat cell is apparently accomplished by increases in both the lipid and cytoplasmic compartments,

but the rate of cytoplasmic growth has proved difficult to quantify. Most investigators have attempted to measure cellular protein, on the assumption that protein forms a constant portion of the cytoplasmic mass, but collagenase, albumin and stromal proteins interfere with the results (Rodbell, 1964b; Goldrick, 1967a). Martinsson (1968) and Englhardt et al. (1971) have avoided the whole problem, of contamination by foreign protein, by measuring cell water and "lipid-free residual space," respectively, as estimates of cytoplasmic mass. Salans and Dougherty (1971) have taken a different approach and have attempted to quantify the degree of collagenase and albumin contamination by use of isotopic labels. The results obtained by the various methods have, however, with one exception (Salans and Dougherty, 1971), been qualitatively similar in that more "cytoplasm," as well as lipid, was found in larger cells. All investigators have agreed, however, that the ratio of lipid to the cytoplasmic index increased as the cell enlarged. This was not solely an age effect, and was observed for selected glycolytic enzymes as well as for total protein (Englhardt et al., 1971; Bjorntorp and Sjostrom, 1972).

Surface area is another obvious size-dependent component and will increase as the cell enlarges. However, if the adipocyte is considered to be a sphere in vivo, an

assumption which Reh (cited by Goldrick, 1967a) has justified, then the surface area/volume ratio of the cell will decline as the cell grows in size. This ratio is one considered critical by Goss (1964) to the efficient operation of any functional biological unit. Since most, if not all, of the hormonal receptor sites are believed to be membrane-bound, surface area has been attributed particular significance by several workers (Zinder and Shapiro, 1971; Hartmann et al., 1971; Bjorntorp and Sjostrom, 1972; Manganiello and Vaughan, 1972) in explaining the metabolic response to cellular enlargement.

As the cell enlarges, then, increases occur in the absolute amounts of lipid, cytoplasm, surface membrane and enzymes in the cell. These changes could undoubtedly account for the increased absolute capacity of the large cell to deposit and mobilize fat. Simultaneously, however, decreases occur in those critical ratios which normally provide for integrated control in the cell. The surface area/volume ratio, the protein/lipid ratio, the enzyme/lipid ratios and the DNA/cytoplasm ratio all decline as a function of cellular growth. A limited capacity of the cell to produce new membrane receptors has been proposed (Zinder and Shapiro, 1971; Hartman et al., 1971; Manganiello and Vaughan, 1972) as one possible explanation for the hormonal insensitivity and reduced metabolic effi-

ciency of large cells, but a decline in the cytological control ratios could provide an equally acceptable explanation. The quantitative relationship between the critical ratios and the metabolic capacity of the cell have not yet been investigated. Age, cell size and nutritional and hormonal stimuli should be tested for their effects upon these ratios in relation to metabolism.

In order to understand more fully the significance of these various relationships to total body function, the variability and adaptability of the total fat cell complement must be examined.

Adipose Growth as a Function of Age

Organs and tissues, in general, exhibit a decrease in proliferative capacity and regenerative potential with advancing age, but they vary in the stage of development at which this declining response becomes evident (Enesco and Leblond, 1962; Strehler, 1962; Goss, 1967; Buetow, 1971). A large degree of variation also exists between individuals, and between species, possibly as a direct reflection of the life-span and metabolic rate (Buetow, 1971). As long as the capacity to proliferate persists, however, there is a reduced reliance upon hypertrophy in the response to functional demands (Goss, 1967). Strehler (1962) has proffered, as one possible explanation for the aging response, the gradual ac-

cumulation of harmful metabolic side-products as a result of perpetual molecular turnover. In renewing tissues, the molecular turnover would occur primarily in the germinal cells, but in non-mitotic tissues, each cell would be responsible for its own molecular renewal. In extensive studies with Sherman rats, Enesco and Leblond (1962) used total DNA of whole body, organs and tissues, to determine the pattern of hyperplasia during growth. As expected, the rate of proliferation was greatest during the early prenatal period, and then showed an exponential decline until 13 to 14 weeks of age. From birth until 13 weeks of age about 4.5 new "generations" of cells were formed in the body as a whole, the rate of proliferation being most rapid prior to weaning.* The weight of body matter (both intracellular and interstitial), per single nucleus, was relatively constant until weaning, and then increased in most organs and tissues, and particularly in tissues such as heart muscle, striated muscle and epididymal fat, which are considered essentially non-mitotic as mature cells. From weaning until 14 weeks of age, 90 per cent of the growth of the epididymal fat mass was attributable to an 18-fold increase in the weight per nucleus. Studies of other fat depots (Zingg, Angel and Steinberg, 1962; Peckham,

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*In rats, the prenatal period is about 23 days in duration. Weaning occurs at 21 days of age and sexual maturity at 7 to 8 weeks of age. The life-span is approximately 1.5 years.

Entenman and Carroll, 1962), using total DNA as an index, have revealed a similar pattern of growth.

Since these early studies, methods for obtaining fat cells essentially free from stromal contamination have been developed (Rodbell, 1964a; Hirsch and Gallian, 1968) and used to study growth changes in adipose tissue. Hirsch and Han (1969) studied changes in epididymal and retroperitoneal fat pads of Sprague-Dawley rats through ages six to twenty-six weeks. At six weeks of age, the pads contained one-third to one-half of their adult complement of mature fat cells. Growth from six to fifteen weeks was accomplished by increases in both cell size and number, but beyond the fifteenth week of life, the depots grew exclusively by the process of cellular enlargement. Similar results have been obtained with the lean Zucker rat (Johnson, et al., 1971) and with Charles River rats (Hubbard and Matthew, 1971). In Swiss Albino and NCS/R mice, the same pattern has been observed (Johnson and Hirsch, 1972); epididymal fat pads grew by size and number increase until 21 days of age (weaning) and thereafter by increases in cell size only.

Data for human adipose tissue is scarce, but the evidence available suggests a similar pattern to that observed in the rat. Hirsch, Knittle and Salans (1966), Brook (1970) and Bonnet et al. (1970) have obtained biopsy samples from a total of 71 normal infants, children and adolescents. On

the basis of cell size or number determinations, these workers have extrapolated to the total body adipose mass and have concluded in favour of a progressive cellular increase to the normal adult quota. Maximal values for cell number were not attained until adolescence or early adult life (Hirsch, Knittle and Salans, 1966). Bray (1970), however, states that the age at which humans stop proliferating fat cells, if this in fact occurs, is not known.

In the animal species previously mentioned, cell size made by far the greatest contribution to total adipose growth. In an attempt to quantify the respective contributions of cell size and number, Di Girolamo and Mendlinger (1971) compared the epididymal fat pads of Wistar rats, hampsters and guinea pigs at six weeks and one year of age. For the rat and the hampster, 81 and 83 per cent of the total weight increase was attributable to cell size, and only 12 and 8 per cent, respectively, to cell number. The situation in the guinea pig was completely reversed with only 6 per cent of the increase attributable to hypertrophy, while 80 per cent was the result of hyperplasia. This information clearly indicates that species differences in the pattern and strategy of adipose growth may exist.

Adaptive Capacity of Adipose Tissue as a Function of Age

In an attempt to modify the attainment of the normal

adult fat cell complement, brief acute starvation and refeeding, prolonged semi-starvation, induced hyperphagia, and prolonged cold exposure have all been applied experimentally to animals during periods of growth. Although most of these studies have used young rats, six to eight weeks of age, in which the number of mature fat cells is still increasing, the experimental results have largely been negative. The only stimulus which has been found effective in limiting the final adult quota of cells in rats has been nutritional deprivation during the preweaning period (Knittle and Hirsch, 1968). In this study, the caloric intake of the suckling rats was varied by manipulating litter size. Thereafter, feeding was ad libitum. The rats which were raised in the larger litters had lighter body weights and pad weights, and smaller and fewer cells throughout the 20 weeks studied. To date, no other stimuli have been tested during this early critical period.

In contrast to these early nutritional effects, 5°C cold exposure, from weaning until 14 weeks of age, has been found to increase the total number of fat cells in epididymal pads of rats (Therriault, Hubbard and Mellin, 1969). In the early weeks, the acquisition of cells was delayed, relative to the controls, but as body weight increased, cell number became significantly greater in the cold-exposed animals. In a study with prolonged semi-starvation (half

rations) from four until 20 weeks of age, Hubbard and Matthew (1971) found a similar delaying effect upon the appearance of mature cells, but the eventual cell quota was normal.

Brief, acute starvation, of a few days to one week in duration, did produce decreases in cell number (Goldrick, 1967; Hirsch and Han, 1969) and DNA synthesis (Hollenberg, Vost and Patten, 1970) in rats seven to nine weeks of age, but the effect was transient and cell number returned to control levels after three to four weeks of ad libitum feeding. These results concur with the general suppression of mitotic activity which Blumenthal (1950) has observed in response to acute starvation. Cell size and lipid content were both greatly reduced by the starvation, but were replenished completely upon refeeding.

Similarly, cell number has proved immune to the effects of caloric excess or weight loss. When hyperphagia was induced by hypothalmic lesion in either seven week old rats (Hirsch and Han, 1969) or adult rats and mice (Hirsch and Han, 1969; Johnson et al., 1971; Johnson and Hirsch, 1972), cellular hypertrophy became extreme, but the number of cells was not altered. Also, weight loss in both humans (Hirsch and Knittle, 1970; Bray, 1970; Brook, 1970) and rats (Hirsch and Han, 1969) was not accompanied by any reduction in the cell complement. Cell size, however, was

diminished drastically. In a longitudinal study with non-obese adult prisoners, Salans, Horton and Sims (1971) were able to induce mild obesity by inactivity and high caloric intake over a period of three to four months. Subsequent dieting and exercise returned the body weight to initial levels. Cell number was not susceptible to modification in either situation, so all changes in body fat were attributable to cell size adjustments. Bjorntorp and Sjostrom (1971) obtained essentially similar results in response to dieting in obese adults.

Physical activity and training have been found to restrict or reduce fat cells to a stable size in growing rats (Palmer and Tipton, 1972) and in sedentary men (unpublished work cited by Bjorntorp and Sjostrom, 1971), but no reports have yet appeared on the effects of early training upon cell number. Mayer (1968) has suggested that physical activity is required for proper regulation of caloric intake, since inactivity leads to metabolic obesity.

In several strains of genetically obese mice, Johnson and Hirsch (1972) have observed extensive cellular hypertrophy but no elevation of the fat cell complement, whereas, in other mutants, the size and number of cells was raised. Similar observations in humans (Hirsch and Knittle, 1970; Bray, 1970; Brook, 1970; Bonnet et al., 1970) have led to the classification of two distinct types of obesity

(Hirsch and Knittle, 1970; Bjorntorp and Sjostrom, 1971). Early onset (hypertrophic - hyperplastic) obesity is characterized by a greater than normal complement of cells and is believed to be determined by patterns established during the early development period. Late-onset (hypertrophic) obesity results solely from an increase in cell size and can only lead to moderate degrees of obesity.

The observation, that hypertrophic factors can only result in moderate degrees of obesity, implies that cell size may be self-limiting. Indirect support for this concept has been derived from several sources: from metabolic considerations (Flatt, 1970; Smith, 1971b; Zinder and Shapiro, 1971); from critical ratio considerations (Goss, 1964); from limits imposed by the distensibility of the cytoplasmic rim (Wasserman, 1965); and from phylogenetic data which have shown a logarithmic relationship between species weight and size trends of functional units (Goss, 1964). The most convincing evidence, however, has come from studies in which a cross-section of obese adult humans have been compared and correlations drawn between cell size and per cent overweight. In these studies (Liebelt, 1963; Preiss, 1967 (cited by Englhardt et al., 1971); Hirsch and Knittle, 1970; Bjorntorp and Sjostrom, 1971), patients who were less than 50 to 70 per cent overweight had a normal cell number, but revealed much enlarged cells. Patients

who exceeded 150 to 170 per cent of ideal weight did not have larger cells than the less-obese subjects, but instead, possessed an additional complement of cells. The reasons for the excess cell quota in extreme cases of human obesity are presently the subject of much research. In this 'maximal' state of cell hypertrophy, mean cell size in obese humans has been reported to be two to three times larger than in control subjects (Bray, 1970; Englhardt et al., 1971), whereas in rats with hypothalamic lesions, a seven to nine-fold increase has been observed (Hirsch and Han, 1969). If, then, a maximal cell size does exist and if cell number is normal and not inducible beyond late adolescence or early adult life (Hirsch and Knittle, 1970), only moderate obesity (up to 50 to 70 per cent overweight) could result from maximal hypertrophy of the existing complement.

Accompanying the change in cell diameter is a change in the shape of the cell size distribution. In obese humans as compared to normals (Bonnet et al., 1970), and in older rats, when compared with younger ones (Di Girolamo and Mendlinger, 1971), the size distribution was found to be more heterogeneous. In the obese humans and older rats, the standard deviation of diameter was larger, but the coefficient of variation (C.V. = $\bar{X} \div$ S.D.) was less. The implications of this effect are not obvious.

In summary, then, studies have clearly indicated

that fat cell size is a very labile component of adipose mass, markedly responsive to nutritional and metabolic stimuli. Adipocyte number, however, is remarkably stable and only a long sustained stimulus, begun very early in life, has been shown effective in altering the complement of adult fat cells. As would be expected, the state of physical training is associated with a reduced fat cell size (Palmer and Tipton, 1972), but the effect of a training stimulus upon cellular proliferation has not been studied. Training, however, like cold exposure and starvation, can create a considerable energy requirement and can invoke considerable hormonal adaptation. Consequently, if applied for prolonged periods sufficiently early in life, an effect on cell proliferation may be demonstrable.

CHAPTER III

METHODS AND PROCEDURES

Sixty-three male Wistar rats (Woodland Farms, Guelph, Ontario) were used in the study. The rats were approximately seven weeks of age at the time of arrival and weighed 160-180 grams. They were immediately placed in individual self-cleaning cages in a temperature controlled room with a 12 hour light - dark cycle (8 a.m. - 8 p.m.). City water was made freely available, and food pellets were allocated twice weekly throughout the study. The food was standard rat chow (Rockland Complete Rat Diet) with the composition: 24 per cent protein, 4 per cent fat and 6 per cent fiber.

On the basis of body weight, the rats were placed into blocks. Matched groups were formed by randomly assigning one rat from each block to each treatment group. The variability in weight within each group, and hopefully genetic pre-disposition to obesity, was thus essentially similar at the start of the study.

The nine rats in Group 1 were sacrificed at this time to provide information regarding initial levels. The remaining six groups were treated as illustrated in Figure 3.1.

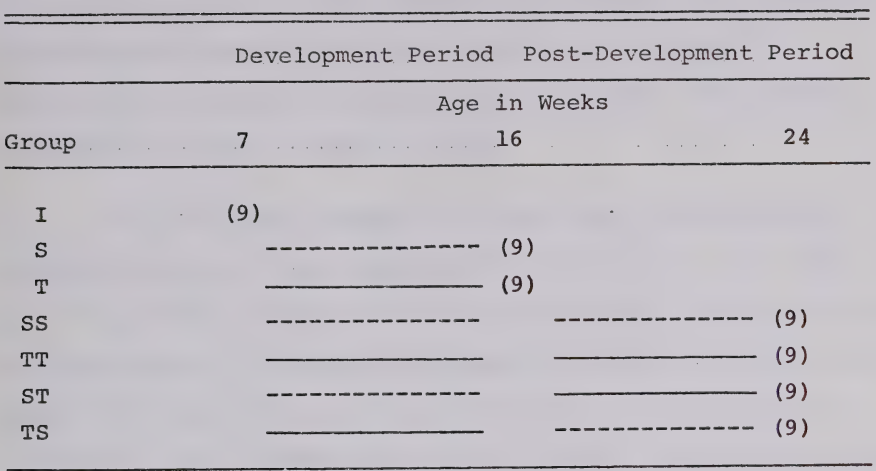

FIGURE 3.1 FORMAT OF THE STUDY

S = sedentary; T = training; I = initial group. Numerals in parentheses represent the number of rats sacrificed.

The first eight week period of the study, until the critical age of 16 weeks, was intended to represent a developmental period during which the genetic potential for cell proliferation may be susceptible to external environmental influences. One trained group (T) and one sedentary group (S) were sacrificed, and their pads examined, at the end of this first period.

The second eight week period, from 16 to 24 weeks of age, was a post-development period during which only cell size should still be subject to change. The remaining four groups were sacrificed at the end of this time. Of these

four groups, Group TT was continuously trained and Group SS continuously sedentary for the whole of the 16 weeks. Group TS was trained and then detrained, while Group ST was trained only after 16 weeks of age.

The training programs, in each case, consisted of endurance running, once each day for five days of the week, on one of two motor-driven treadmills. The speed and duration of the training was increased progressively over the first three weeks and then maintained constant at one mile per hour for one hour. The rats were able to run steadily at this workload after three weeks.* The training was facilitated by means of an electrical shocking device at the back of the treadmill.

All rats in the exercising groups were fed ad libitum throughout their respective training periods. Sedentary rats, however, were observed to eat more than training rats, so rats in the sedentary groups received a restricted ration corresponding to the average amount eaten by the rats in the training groups. By equilibrating the diets of these control groups with those of the training rats, spurious effects due to differences in caloric intake could be largely controlled.*

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*This program has been used extensively and is reported in the literature (Gollnick et al., 1969) to ellicit intensive glycogenolytic and lipolytic responses in rats. After eight weeks at this pace, rats can run from one to four hours before fatigue.

*An alternative procedure (Palmer and Tipton, 1972) involves pair-weighting of rats; i.e., restrictive feeding of each sedentary rat so that body weight remains equal to that of the training partner. This procedure, however, could restrict the natural growth patterns of other organs and tissues besides that of adipose tissue.

The only exception to the feeding pattern was Group TS, which was fed ad libitum during the detraining period in order to maximize the post-development stimulus to cell hypertrophy and proliferation.

At the time of sacrifice, the rats were killed with an overdose of ether, weighed on a basket balance and the weight recorded. The entire left and right epididymal fat pads were surgically removed by dissecting free from the head of the epididymis and by cutting the major supplying blood vessels. Each pad in turn was placed in rat Ringer solution in a teflon saucer and the remainder of the major blood vessel was dissected out. The pad was then thoroughly rinsed in clean Ringer solution, blotted on a clean paper towel, placed on a tared polyethylene boat and weighed on a Mettler electric balance. The pad was returned to Ringer solution and the weight recorded. The left and right fat pads from the same animal were found to vary by as much as 20 per cent, so the larger of the two pads was always used for the subsequent analysis. The only exception to this was Group I, in which both pads were required to provide sufficient tissue.

The pad to be analysed was placed in clean Ringer solution in the teflon dish and minced into pieces of less than 25 mg. using a microdissection needle and scissors. This procedure was deemed necessary in order to ensure more

representative sampling of the cells in the tissue. Pieces could be removed at random from the mince to obtain the desired quantity of tissue for determination of cell size and lipid content.

Electronic Counting and Sizing of Collagenase-Liberated Cells

Initial attempts were made to use the Coulter electronic counter (Model B, Coulter Electronics, Hialeah, Florida), since Hirsch and Gallian (1968) and Sjostrom, Bjorntorp and Vrana (1971) have successfully used this instrument for counting and sizing fat cells. These investigators used osmium tetroxide fixative to liberate the fat cells and Hirsch and Gallian (1968) employed nylon mesh sieves to trap the cells and remove the stromal elements. The extreme expense of the osmium fixative, however, prohibited its use in this study and the 25μ mesh screens could not be obtained.

An alternative procedure was tried in an attempt to obtain uncontaminated suspensions of free fat cells. These procedures (Rodbell, 1964a) involved collagenase digestion of tissue, mild centrifugation to float the liberated fat cells, repeated washing with buffer and aspiration of the infranatant buffer containing the sedimented stromovascular elements. The fat cells were then chilled at $4^{o}C$ and fixed with glutaraldehyde-trichloroacetic acid as outlined by

Hirsch and Gallian (1968). These methods, however, resulted in considerable breakage. Also, a subsidiary peak was always present at small cell sizes (less than 40μ) in the frequency distribution. This peak, whether due to stromal contamination, air bubbles or lipid droplets, could not be avoided, so other methods were sought for counting and sizing of cells.

Determination of Fat Cell Size

Adipocyte size was determined on a preparation of isolated fixed cells using a microscope.

Suspensions of free fat cells were prepared by collagenase digestion of tissue pieces, essentially as described by Rodbell (1964a). Samples of 300 to 400 mg were obtained by random sampling of pieces from the tissue mince. The pooled tissue pieces were placed in a plastic incubation vial in a metabolic shaker at 37°C and 80 cycles per minute for one hour. The vial contained 3 ml Krebs-Ringer bicarbonate buffer with half of the normal complement of calcium (Rodbell, 1964a), 3μM glucose per ml, 40 mg albumin per ml (Baker, bovine serum albumin, Fraction V), and 3 mg bacterial collagenase (Sigma, Cl. histolyticum Type 1, Lot 81 C - 0080). The contents of the vial were brought to a final pH of 7.4, with 0.1N NaOH, for optimal collagenase activity for optimal collagenase activity (Rodbell, 1964a). The reduced complement of calcium was necessary to prevent precipitation.

After incubating for one hour, the vial was removed. Large pieces of tissue still remained at the surface and no amount of shaking or swirling was effective in releasing all the cells. Almost quantitative release of cells could be achieved, however, by repeatedly moving the incubate in and out of a short-nosed, siliconized Pasteur pipette. The cell breakage caused by this procedure was not extensive, as evidenced by the absence of large lipid droplets at the surface of the medium.

The suspended cells were then washed by adding 10 ml cold buffer and swirling. The vial was allowed to stand undisturbed for five minutes to float the free fat cells. The infranatant was then removed by aspiration through a siliconized Pasteur pipette using suction from a rubber bulb. An additional 10 ml albumin-free buffer were added and the vial was placed at $4^{o}C$ to chill the cells.

After one hour at $4^{o}C$, the infranatant was again removed by aspiration and the chilled cells were fixed in an aqueous solution containing 0.25 per cent trichloroacetic acid and 6.25 per cent glutaraldehyde, as outlined by Hirsch and Gallian (1968). Ten ml of the fixative solution were added to the chilled cells in the vial and after gentle swirling, the vial was returned to $4^{o}C$ for one hour.

After fixation for one hour, the vial was removed from the cold. A siliconized short-nosed Pasteur pipette was then

used to transfer several drops of the thick suspension of floating cells to a small vaseline containing - ring on a siliconized glass slide. Two small drops of methylene blue were added. The strained suspension was then inspected under the low power (50X magnification) of a Vickers microscope and was adjusted, by aspiration and by the use of Ringer solution, until the drop was slightly convex and the cells appeared as a dispersed single layer. Cell sizing was then carried out at 400X using a previously calibrated ocular micrometer. A size distribution was obtained by grouping the cells into intervals of approximately eight microns. In this way, 300 cells could be sized in one hour. The size distribution was used to calculate mean cell diameter in the conventional manner (Appendix A).

Determination of Mean Cell Volume

Mean cell volume was calculated from the diameter data using the limits of the size intervals instead of the midpoints. This procedure was proposed by Di Girolamo, Mendlinger and Fertig (1971) to take into consideration the nonlinear transformation involved in converting diameters to volumes ($V=\pi D^3/6$). Thus, all cells falling within a class interval were assigned a volume equal to the average of the smallest and largest volumes in the interval. Subsequent steps for the calculation of mean cell volume are outlined in Appendix A.

Determination of Average Size Distribution

An average size distribution was obtained for each

group by pooling all of the size frequency data for all the rats in the group. In this way, a mean frequency was obtained for each size interval. The mean diameter and standard deviation of this pooled size distribution was plotted as a percentage frequency distribution to permit more meaningful comparisons between groups.

A surface area transformation was made on this data in the same way as the volume transformation above ($S.A=\pi D^2$).

Determination of Weight of Lipid Per Cell

The mean lipid weight per cell was calculated for each rat from the mean cell volume by assuming a constant lipid density (0.915) equal to that of triolein. Thus,

$$\overline{M} = \overline{V} \times 0.915 \times 10^{-3}$$

(g/cell) (pl)

This calculation makes the assumption that the measured cell diameter is that of the lipid droplet of the cell, and that the cytoplasmic rim is negligible.

Determination of the Lipid Content of the Tissue

Lipid content of the fat tissue was determined as carboxyl ester content by the methof of Rapport and Alonzo (1955) after extraction in an isopropanol-heptane system (Dole and Meinertz, 1960).

Forty ml of an isopropanol : heptane mixture prepared according to Dole and Meinertz (1960), were placed in a Vertis homogenizing flask together with 8 ml of rat Ringer solution. A random sample of approximately 100 mg of minced pad segments was placed in the flask and homogenized for five minutes for extraction of the lipids. Sixteen ml. of distilled water and 24 ml. of heptane were added to induce the most effective separation of the phases. After shaking and standing for 10 minutes, five 0.2 ml aliquots of the upper heptane phase were placed in clean test-tubes and evaporated in a boiling water bath until only an oily residue remained (approximately two hours). From this stage, each test-tube was treated individually and the carboxyl ester content was determined colourimetrically by the hydroxylamine method of Rapport and Alonzo (1955). The ferric perchlorate required by this method was not available commercially, so it was prepared as outlined by Skidmore and Entenman (1962). Standards were run with each batch of samples. Optical density readings were obtained on a Bausch and Lomb Spetronic 20 spectrophotometer and a mean for the five aliquots was calculated. The lipid concentration was determined from the mean by using a regression equation developed from standard solutions of tripalmitin. By extrapolation from the aliquot to the original sample weight, an estimate of the lipid content of the tissue was obtained (see Appendix A).

Determination of Cell Number

The total number of cells in the larger of the two epididymal pads was determined indirectly for each rat from the data on lipid contents of the pad and of the mean cell. Thus,

Cell number = total lipid in pad (mg)x10^3÷µg lipid/cell

Determination of Fatness Index

An approximate index of fatness was derived for each rat, for purposes of this study, by dividing the total weight of epididymal fat in mg (the sum of two pads) by the body weight in grams. Thus,

Fatness Index = mg epididymal fat ÷ g body weight

The assumption inherent in the use of this index is that epididymal fat is a constant proportion of total body fat.

Statistical Analysis

Group means and standard errors were calculated for all of the variables investigated in the study. A randomized group design analysis of variance was performed on each of the seven major variables and a Newman-Keuls range test was used to determine the differences between pairs of group means. The seven major variables were: body weight, weight of the larger pad, fatness index, mean cell diameter, µg lipid per cell, pad lipid content (%) and cell number.

The differences between the group means for each variable were considered significant at alpha less than 0.05 and probability values were reported.

CHAPTER IV

RESULTS

The raw data for all of the groups are listed in Tables B1 to B3 of Appendix B. The mean and standard error for each group on each variable appears in Table 4.1 and group comparisons are presented diagrammatically in Figures 4.2 to 4.7. Figure 4.8 depicts the average frequency distribution of diameters (Table B4) for each group. The group growth curves are plotted in Figure 4.1 from data in Table B1.

When the data was subjected to an analysis of variance, significant F ratios ($p < .001$) were obtained for all seven variables: body weight, weight of the larger fat pad, Fatness Index, mean cell diameter, cell lipid content, per cent lipid in pad and cell number. The analysis of variance summary tables appear in Appendix D.

Significant differences between pairs of means, as determined by the Newman-Keuls comparison procedure, are summarized in Table 4.2. The group sacrificed initially (Group I) was found to be significantly different ($p < .01$)

TABLE 4.1 VARIABLE MEANS ± STANDARD ERROR OF THE MEAN

Group	Body Weight (gm)	Largest Pad Weight (mg)	Fatness Index (mg/g)	Mean Cell Diameter (μ)	Mean Cell Volume (pl)	Lipid Per Cell (μg)	Pad Lipid Content (% Wt)	Surface Area (μ^2) ($\times 10^3$)	Cell Number ($\times 10^6$)	SA/Volume Ratio (μ^2/pl)
I	201.1 ± 3.8	524.7 ± 28.6	5.04 ±0.22	55.95 ± 1.08	105.2 ± 5.8	0.096 ± .005	66.19 ± 1.85	10.4	3.50 ±0.126	98.9
S	447.2 ± 7.5	3550.9 ± 218.8	15.45 ±0.99	90.00 ± 1.40	417.5 ±19.2	0.382 ± .018	83.92 ± 2.82	26.1	7.79 ±0.395	62.5
T	382.4 ±13.2	1864.3 ± 156.8	9.43 ±0.59	68.94 ± 1.54	191.0 ±13.2	0.175 ± .012	79.39 ± 4.20	15.4	8.44 ±0.532	80.6
SS	443.2 ±10.8	4055.7 ± 291.2	17.61 ±1.16	92.41 ± 2.03	454.5 ±28.0	0.416 ± .026	80.22 ± 3.95	27.4	7.91 ±0.605	60.3
TT	437.6 ±16.3	2217.2 ± 159.2	9.94 ±0.62	72.35 ± 1.90	223.0 ±17.2	0.204 ± .016	78.65 ± 2.80	17.1	8.72 ±0.650	76.7
ST	427.1 ± 9.7	2615.9 ± 271.3	11.48 ±1.01	75.21 ± 1.94	248.7 ±18.7	0.228 ± .017	82.02 ± 2.44	18.3	9.34 ±0.724	73.6
TS	505.9 ±10.3	4261.2 ± 421.4	16.05 ±1.40	90.40 ± 2.67	439.6 ±36.3	0.402 ± .033	88.33 ± 3.61	26.8	9.42 ±0.767	61.0

TABLE 4.2 SUMMARY OF MULTIPLE COMPARISONS

Groups	Body Weight (gm)	Largest Pad Weight (mg)	Fatness Index (mg /g)	Mean Cell Diameter (μ)	Lipid Per Cell (μg)	Pad Lipid Content (% Wt)	Cell Number ($x10^6$)
I - S	**	**	**	**	**	**	**
I - T	**	**	**	**	**	**	**
I - SS	**	**	**	**	**	**	**
I - TT	**	**	**	**	**	**	**
I - ST	**	**	**	**	**	**	**
I - TS	**	**	**	**	**	**	**
S - T	**	**	**	**	**	-	-
S - SS	-	-	-	-	-	-	-
S - TT	-	**	**	**	**	-	-
S - ST	-	-	*	**	**	-	-
S - TS	**	-	-	-	-	-	-
T - SS	**	**	**	**	**	-	-
T - TT	**	-	-	-	-	-	-
T - ST	**	-	-	-	-	-	-
T - TS	**	**	**	**	**	-	-
SS - TT	-	**	**	**	**	-	-
SS - ST	-	**	**	**	**	-	-
SS - TS	**	-	-	-	-	-	-
TT - ST	-	-	-	-	-	-	-
TT - TS	**	**	**	**	**	-	-
ST - TS	**	**	**	**	**	-	-

α = 0.05 *

α = 0.01 **

from all other groups on all seven variables tested. In almost all cases, also, groups which were training at the time of sacrifice were significantly different from those which were sedentary in terms of fat pad weight, Fatness Index and cell size. No differences were found between any groups (except Group I) with regard to the number of cells or the per cent lipid in the pad.

Body Weight and Fatness Index

The body weights obtained for the rats at each week during the study were averaged for each group. The mean body weights at each week are presented in Table B1, and weight progress is depicted graphically in Figure 4.1. Figure 4.2 is a block diagram of the group mean weights at the time of sacrifice. All sedentary rats had received restricted rations, throughout the study, equivalent to the average amount eaten by the exercising rats. Only the detraining rats received food *ad libitum* while they were sedentary.

The Fatness Index obtained for each rat (Table B3) was averaged for each group and these mean values are listed in Table 4.1. A block diagram (Figure 4.3) is also included for visual inspection.

Body weights in all groups showed a progressive

increase throughout the first eight week period of the study. All three training groups (Groups T, TT and TS) had lower mean body weights than did the sedentary groups at this time (16 weeks of age). Group T was significantly lighter than Group S, but Group S had shown a peculiarly rapid rate of growth.

Beyond 16 weeks of age, the growth rate of Group SS slowly declined and had almost plateaued by 24 weeks. Group TT continued its delayed rate of growth and by the age of 24 weeks had reached the weight level of Group SS, despite a significantly lower Fatness Index. The Fatness Indices were 9.94 and 17.61 mg epididymal fat/g body weight, respectively for groups TT and SS.

In the detraining group (Group TS), a very sharp increase in body weight was evident after the onset of sedentary life and of ad libitum feeding. The food intake of these rats appeared to increase during this period.* At 24 weeks of age, the mean body weight of the group was 506 g and was significantly greater than that of all other groups, including Group SS (443 g) which had been continuously sedentary throughout. Group SS, however, had been on restricted rations. Moreover, the weight of Group TS was

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* Only crude estimates of food intake were obtained, so data are not presented. The food eaten was not measured for each rat, but only for the group as an average. Droppings were not collected.

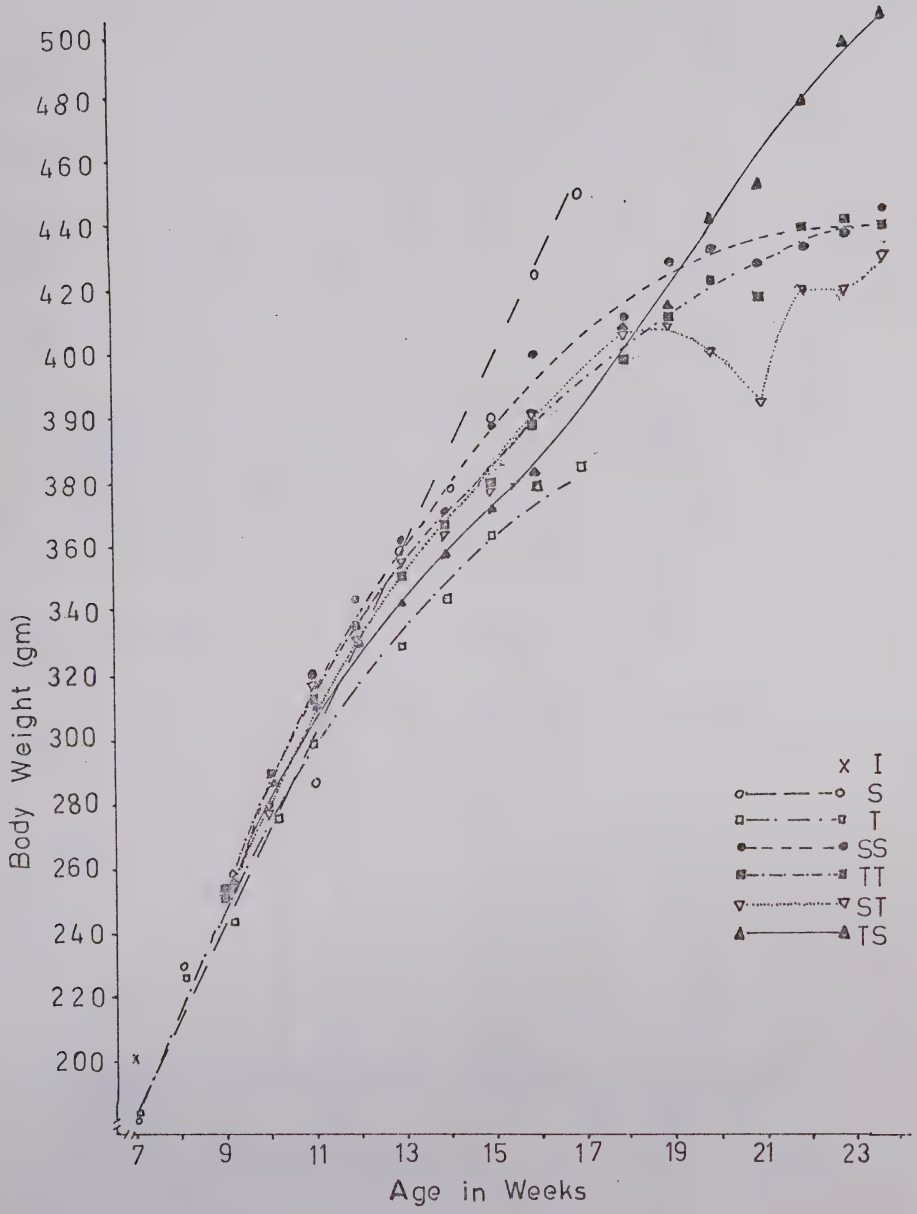

FIGURE 4.1 WEIGHT PROGRESS CHART

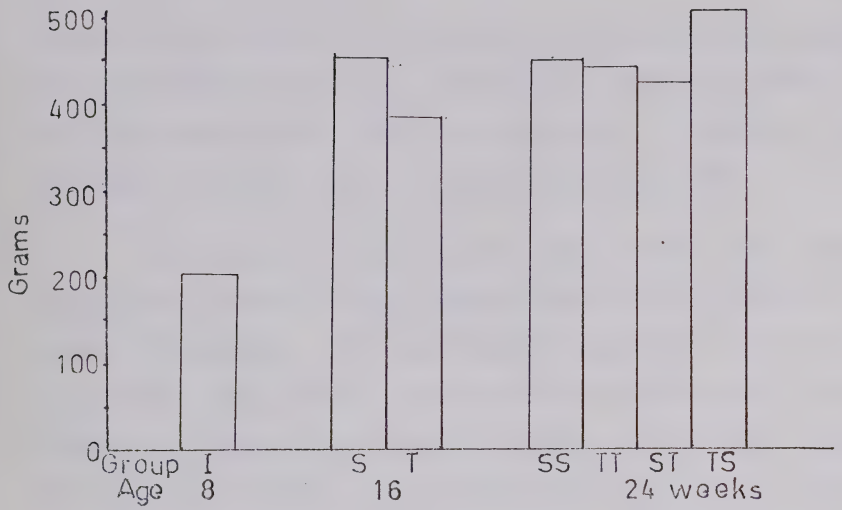

FIGURE 4.2 BODY WEIGHT

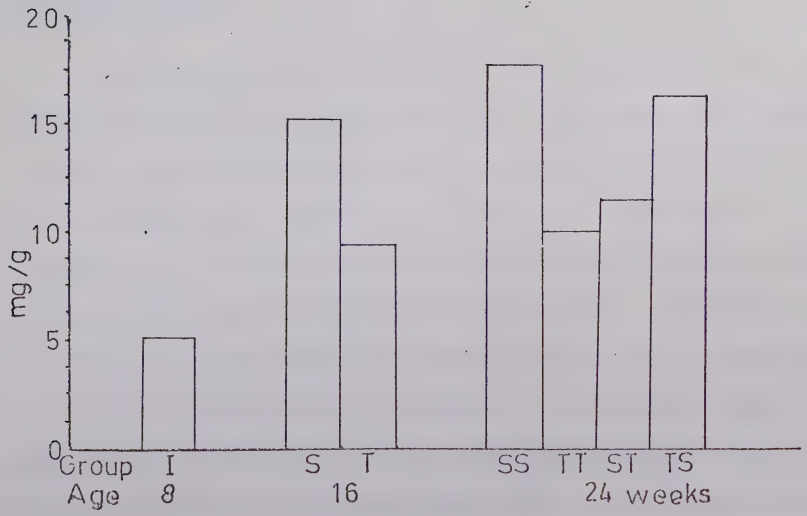

FIGURE 4.3 FATNESS INDEX

still rising rapidly at the end of the study, whereas Group SS had reached a plateau. The Fatness Index rose along with the body weight, but was not greater in Group TS than in Group SS at the time the study was terminated.

In Group ST, the late-training "weight-loss" group, a distinct decline in body weight began shortly after the onset of training. The body weight dropped by 20 g and then rose again within a further two to three weeks. Food intake was observed to drop considerably during the weight loss period. At the time of sacrifice, the Fatness Index was intermediate between those for Groups T and TT and those for the sedentary groups (S, SS and TS).

Fat Pad Weights

Values for both left and right epididymal fat pads of all rats are listed in Table B3. The group mean weights for the larger of the two pads are presented in Table 4.1 and diagrammed in Figure 4.4. All groups which were sedentary at the time of sacrifice had significantly larger fat pads than did those which were training. The only exception was the comparison between Group S and Group ST and this difference nearly reached significance. The fat pads of the late training group (ST) were larger than, but not significantly different from, those of the continuously trained groups. Similarly, neither the sedentary rats at

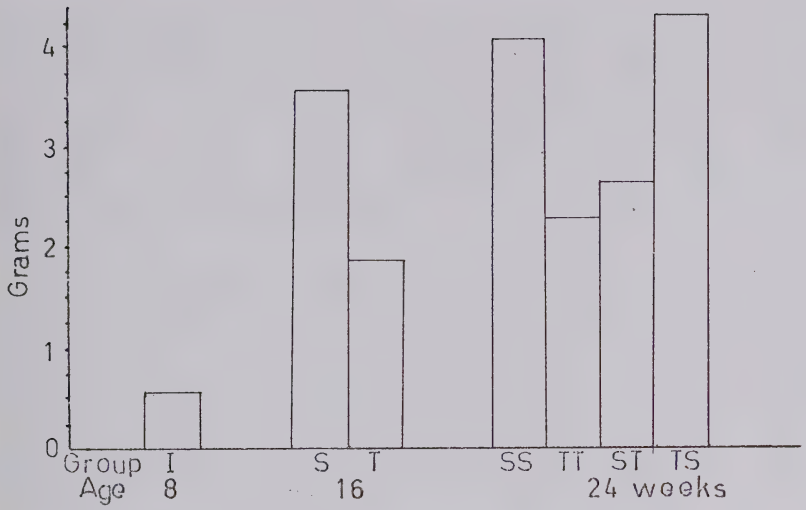

FIGURE 4.4 LARGER PAD WEIGHT

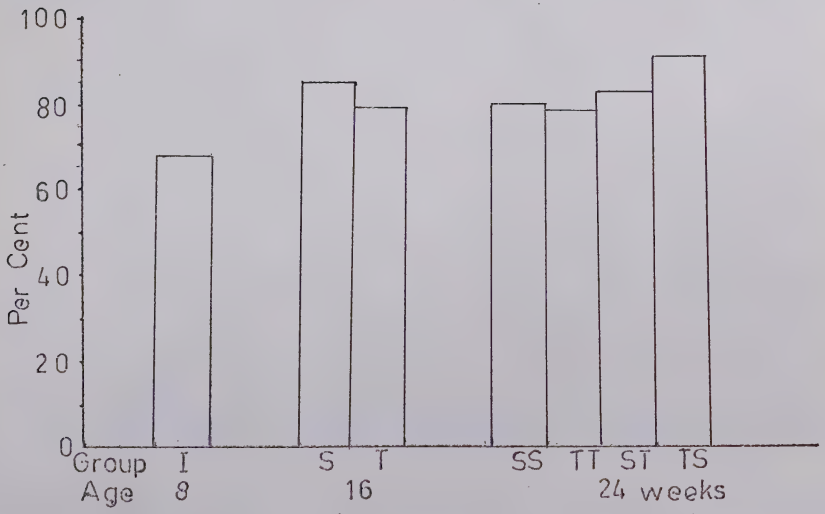

FIGURE 4.5 PAD LIPID CONTENT

16 and 24 weeks, nor the training rats at these times, were different with respect to this variable. The detraining group (TS) had the largest fat pads (4261 mg) of all groups, but the difference was not sufficient to permit significance from other sedentary groups.

The left epididymal pad was found to be larger than the right in six of the seven study groups, in 67 per cent of all rats and in 78 per cent of the training rats. When the difference between the group means for the two pads was expressed as a per cent of the weight of the smaller pad, the differences were ·8, ·4, 3·2, 2:3, 2·4 and 10 per cent respectively for groups I, S, T, SS, TT, TS and ST. When individual animals were examined, the largest difference observed was one of 26 per cent for a rat in Group ST.

Pad Lipid Content

The per cent of pad weight which was lipid was found to be an extremely variable component in the study. Only the initial group (Group I), with a lipid content of 66 per cent, yielded significant differences. In all the other groups, the mean pad lipid content was in the range 79 to 88 per cent (Table 4.1 and Figure 4.5), but the range of variation within each group was about 30 per cent (Table B3).

Cell Size

Diameters of 200 to 300 cells were measured for each

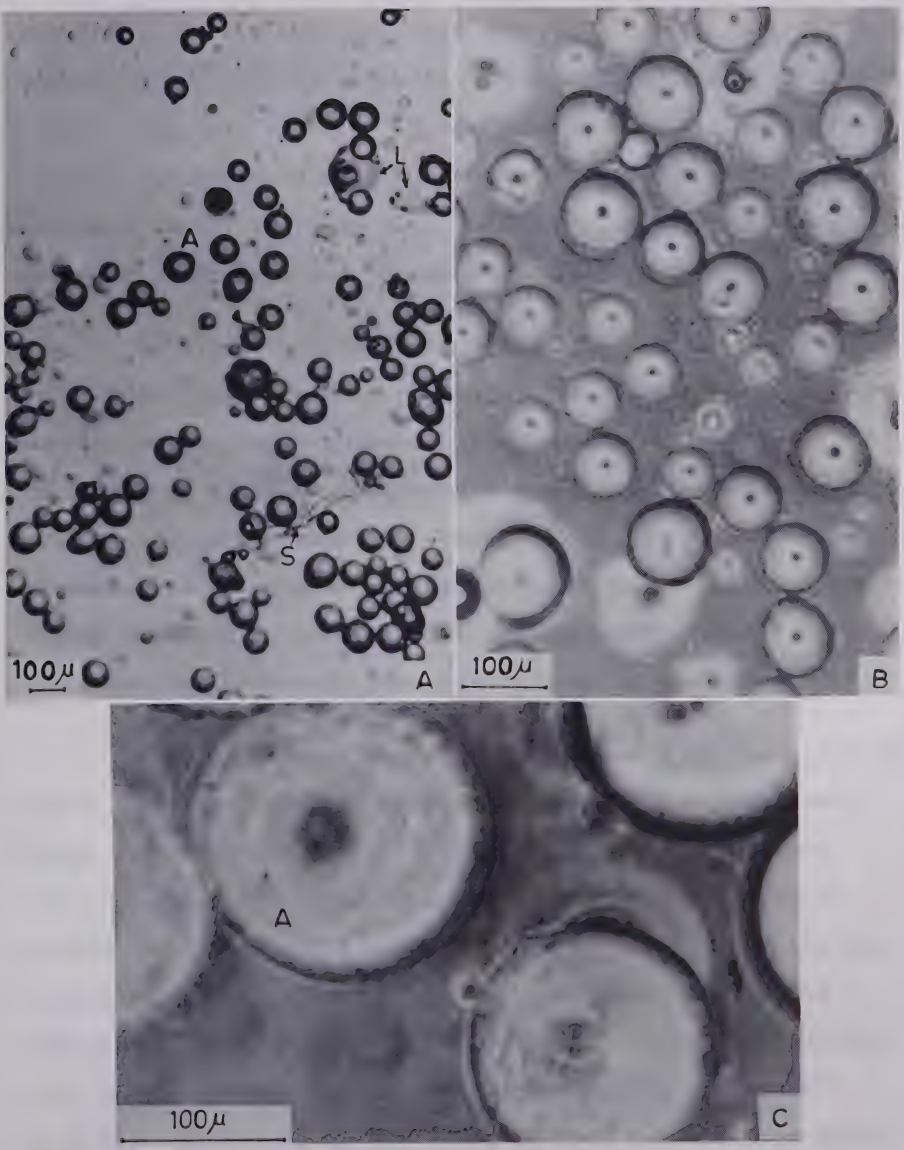

PLATE 4.1 MICROSCOPE SIZING OF FAT CELLS

A. Light microscope X40 showing dark spherical adipocytes (A), stromal fibres (S), and pale lipid droplets (L)
B. Phase objective X100
C. Phase objective X400

rat (Plate 4.1). The frequency distributions of cell diameter appear as raw data in Table B2. Mean cell diameter (μ), mean cell volume (pl) and cell lipid mass (μg) are presented for each rat (± standard error) in Table B3 and for each group (± standard error) in Table 4.1. Cell size, expressed as μg lipid/cell, is depicted diagrammatically in Figure 4.6.

An average distribution of cell diameters was calculated for each group by pooling, in a weighted manner, the individual distributions. These data are tabulated in Table B4 and plotted in Figures 4.8 and 4.9. In these average distributions, frequencies are expressed, for group comparison purposes, as a percentage of the mean number of cells sized. Figure 4.8 clearly demonstrates the normality of cell size distributions when cell diameter is used as the size index.

Figures 4.8 and 4.9 also illustrate a tendency for the group distributions to be clumped in relation to treatment. Group I forms a sharp peak at 56μ distinct from those of other groups. The training groups (T, TT and ST) form an intermediate clump with peaks between 69 and 75μ , while the sedentary groups (S, SS and TS) exhibit more diffuse distributions which peak between 90 and 93μ . The peaks within each of these three clumps were significantly different ($p < .01$) from those in the other clumps, but not from each other (Table 4.3). When these mean cell diameters were sub-

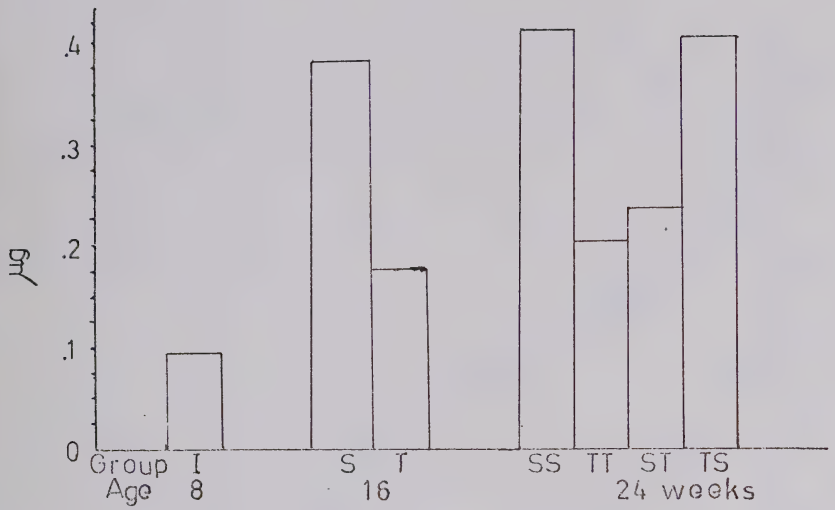

FIGURE 4.6 LIPID PER CELL

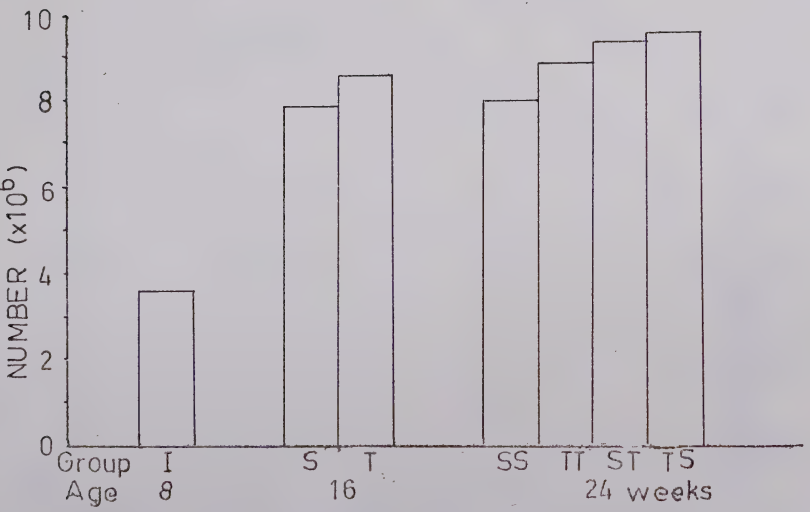

FIGURE 4.7 CELL NUMBER IN LARGER PAD

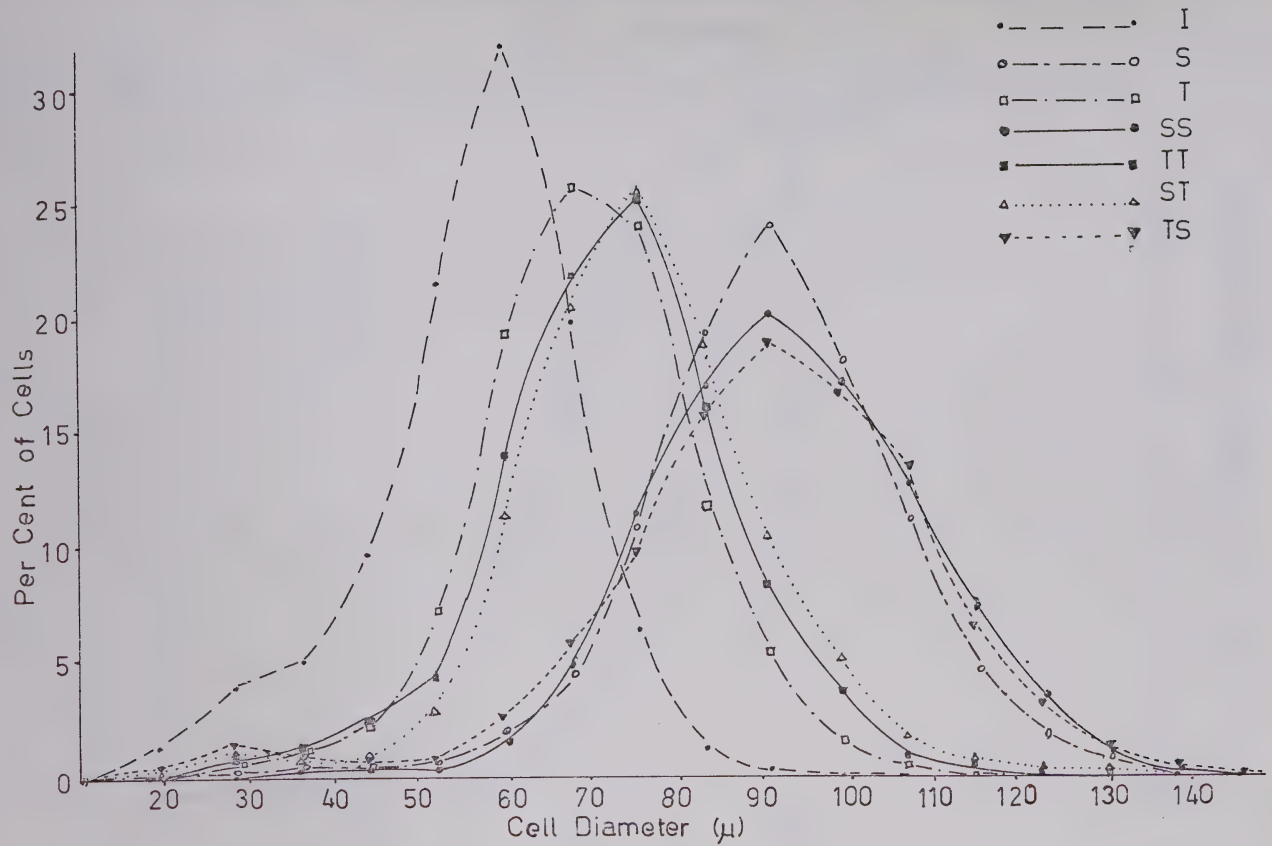

FIGURE 4.8 DISTRIBUTION OF CELLS

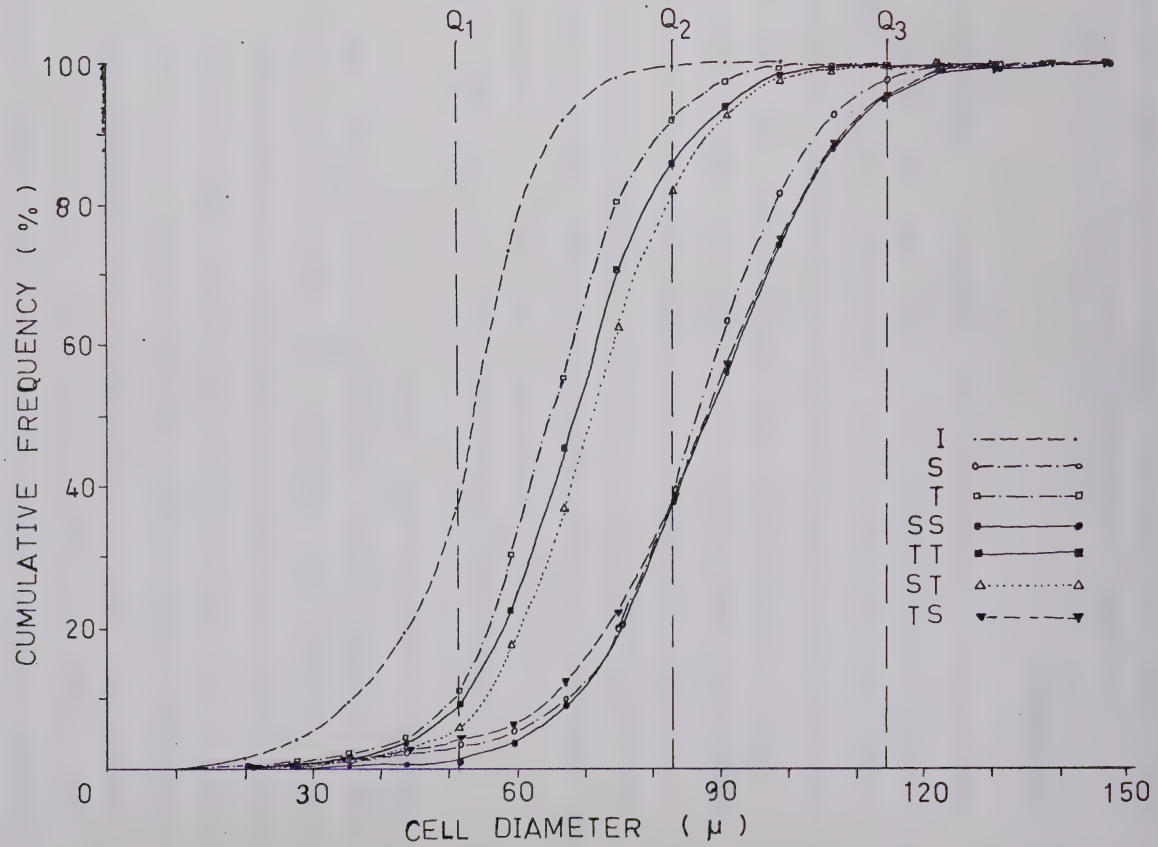

FIGURE 4·9 CUMULATIVE DISTRIBUTION OF CELLS

jected to a volume transformation, the cell volumes for the three clumps were approximately in the ratio 1 : 2 : 4 (Table 4.1). Thus, the fat cells of the sedentary rats contained, on the average, twice as much fat as did the cells of the training rats. The training rats, in turn, had a lipid volume per cell which was twice that of the seven week old rats in Group I. The largest cells observed for the various groups were 100, 114, 130, 130, 140, 150 and 150μ respectively for Groups I, T, TT, ST, S, SS, TS.

The mean surface area per cell, as calculated from the average size distribution for each group, is reported in Table 4.1. The mean surface area/volume ratio for each group is also presented in Table 4.1. The mean surface area/volume ratios are clumped in the same way as the diameters: Group I has a mean ratio of 98.9 μ^2/pl; the three training groups (T, TT and ST) have mean ratios of 80.6, 76.7 and 73.6 μ^2/pl; the three sedentary groups (S, SS and TS) have ratios of 62.5, 60.3 and 61.0 μ^2/pl, respectively. On the average, the three training groups had 25.6 per cent more surface area per unit of contained lipid than did the sedentary groups.

Cell Number

The number of mature fat cells in the larger fat pad is listed for each rat in Table B3 and the mean for each

group is in Table 4.1. Between seven weeks of age (Group I) and 16 weeks of age (Groups S and T) greater than a two-fold increase in cell number occurred ($p < .01$), but beyond 16 weeks, no significant changes were observed. Larger cell complements were recorded for the continuously trained group than for the sedentary group at both 16 weeks of age and 24 weeks of age, but significance was not attained. Wide variations in cell number were observed within each group.

CHAPTER V

DISCUSSION

Effects of Training Upon Growth Rate and Fat Deposition

The progressive body weight gain which occurs during normal growth is the result of both fat accumulation and an increase in the lean body mass. The results of this study clearly demonstrate the capacity of daily endurance exercise to limit fat accumulation in rats during growth. The fat pad weights and Fatness Indices of the continuously training rats were slightly less than half of the respective values for the sedentary groups at 16 and 24 weeks of age. Nevertheless, the Fatness Indices of the training groups (T and TT) were almost twice as high as those determined for seven week old rats. This indicates that fat accumulation occurs at a much more rapid rate than does increase in body weight, even in training animals.

In addition to decreasing the fat pad weights, however, training appeared to produce an increase in the lean body mass. This was evident in two cases: firstly, by the training rats in Group TT, which reached the same body weight as Group SS at 24 weeks despite significantly less epididymal

fat; and secondly by the detraining group (TS) which attained a significantly greater body weight than Group SS at 24 weeks, even though fat pad weights and Fatness Indices were similar in the two groups. Presumably this weight gain, which was delayed relative to the sedentary rats, was due to muscle hypertrophy and deposition of bone salt as a result of the physical activity.

Effects of Training Upon Fat Cell Number

Severe under-nutrition in pre-weaning rats (Knittle and Hirsch, 1968) and sustained cold-exposure (Therriault, Hubbard and Mellin, 1969) in rats of 50 to 75 g (immediate post-weaning) have been found capable of modifying adult fat cell number; the former had a limiting effect while the latter caused an increase. However, no stimuli applied to experimental animals beyond this young age have been found to alter significantly the adult cell quota (Hirsch and Han, 1969; Hollenberg, Vost and Patten, 1970; Hubbard and Matthew, 1971; Johnson et al., 1971). The endurance training stimulus applied to seven week old rats in this study does not appear to be an exception. All of the groups which received some training, whether early, late or continuously, tended to have larger cell complements than the sedentary groups (S and SS), but none of these differences attained significance. These results generally support the conclusions of Goss (1964) and of Enesco and

Leblond (1962) that, beyond the initial growth period, static tissues are very reluctant to compensate for changes in functional load by adjusting the cell numbers. Since nerve is also a static tissue, it appears probable that adipocyte hyperplasia may be limited beyond the neonatal period when innervation has become established.

The results with the various stimuli, then, tend to suggest that the rate of cell proliferation is not subject to modification beyond seven weeks of age, yet the number of mature fat cells present in the pad continues to increase until 15 weeks of age (Hirsch and Han, 1969; Hubbard and Matthew, 1971). Moreover, prolonged semi-starvation (Hubbard and Matthew, 1971) and sustained cold-exposure (Therriault, Hubbard and Mellin, 1969) can delay the acquisition of the full cell quota. Any one of three alternatives could explain these apparent contradictions:

(i) if cell division can continue beyond seven weeks of age, and if the rate of proliferation is modifiable, then the eventual number of cells, or the ultimate number of cell divisions, is self-limiting;

(ii) that the full adult cell quota may be present in the fat pad by seven weeks of age, partly in the undetectable form of immature primordial cells, and that stimuli beyond this age may modify the rate of differentiation, but

not proliferation, of those pre-existing cells;

(iii) that the methods presently available, and the experimental designs possible, are not sufficiently powerful to detect minor changes in cell number.

It is not possible on the evidence available, to elaborate on the first two alternatives, but the second would seem to be the more likely explanation. The third possibility deserves some further comment. Studies with both rats and humans have, of necessity, been of a cross-sectional nature; ie, one rat or one human cannot be sampled throughout growth. In view of this fact, and the large individual variations which appear to exist in the "normal" adult cell complement, an extremely large number of subjects would have to be used to detect small differences in cell numbers. To date such studies have not been conducted.

Nevertheless, it can be concluded that if hyperplasia does play any role in the adaptive expansion of adipose mass, the role is minor relative to the changes which occur in cell volume.

Effects of Training on Fat Cell Size

Cellular hypertrophy in the adipose tissue was found to be effectively limited by the training stimulus, either directly or indirectly, to a volume approximately

half of that found in sedentary rats. Palmer and Tipton (1972) have also found a decrease in fat cell size when Sprague-Dawley rats were trained for 10 - 13 weeks. According to hormonal sensitivity studies on isolated fat cells, this small cell size would accrue upon the trained rat a lesser absolute capacity to mobilize and deposit lipid, but a greater efficiency per unit weight of stored lipid. This efficiency aspect would be an advantage in such activities as endurance running, where weight economy is vital. The observed effects of training upon fat cell size agree with the literature reports which represent cell size variations as being the predominant factor in the fluctuations of adipose mass.

In addition to the differences in mean cell size between the sedentary and training groups, there was also an effect upon the shape of the cell size distribution. In sedentary rats, the distribution was more diffuse, while in the trained rats, the cells were more homogeneous in size. Similar observations have been made by Di Girolamo and Mendlinger (1971) and Bonnet et al. (1971). The method of attainment of the compact size distribution in trained animals, however, is not immediately evident. The distribution differences would not seem to be readily explainable in terms of hormonal sensitivity considerations. One plausible explanation would be the existence in the tissue

of many small compartments of cells (Bonnet et al.,1971), presumably each with its own trophic nerve. The sympathetic discharge with training or other stresses may be sufficient to selectively drain the larger adipocyte groups so that a more homogeneous distribution results.

Effects of Detraining in Adult Rats

The results suggest that Group TS accumulated fat very rapidly after cessation of training at 16 weeks of age. Though the reduced energy output must be the major factor involved, a measured increase in food intake accompanied the onset of sedentary life and must partly account for the large fat pads found in these rats. By 24 weeks of age, when the rats were sacrificed, the Fatness Index in this group was comparable to that of the continuously sedentary group, but the body weight of the detraining rats was still increasing rapidly, whereas Group SS had reached a plateau. However, since the sedentary rats were on a slightly restricted diet, this comparison has only limited significance. The fat pad weights recorded by Taylor, Booth and McBean (1972), for ad libitum fed control animals of the same age, were considerably higher than those obtained in the present study.

The detraining group had been fed ad libitum to maximize the stimulus to fat accumulation, and the results

clearly indicate that the effects of the training program, in limiting adipose mass, can be readily reversed by an excessive caloric stimulus. The number of mature fat cells in the pad was not significantly different from that of any other group, so the large amounts of fat accumulated during detraining must have been deposited, primarily, in pre-existing cells. The mean cell size of 90μ, similar to that of the sedentary rats, attests to this conclusion. A similar excessive cell hypertrophy, without a change in cell number, has been observed in cases of hyperphagia induced either in young or adult rats by gold- thioglucose injection or hypothalamic lesion (Hirsch and Han, 1971; Johnson et al., 1971).

Effects of Training Upon Sedentary Adult Rats

When Group ST was subjected to physical training beyond 16 weeks of age, body weight dropped by 20 g but rose again by 24 weeks as a result of normal growth in lean body mass. Fat pad weights and Fatness Index at the end of the study were intermediate between those of the continuously trained (TT) and continuously sedentary (SS) rats, but only the differences from the sedentary rats were significant. The weight loss, however, could be attributed only partly to the exercise effects, since food intake was also observed to decrease with the onset of training and possibly some degree of stress or trauma was involved.

The reduction which occurred in the fat pad/body weight ratio appeared to be due entirely to changes in the size of the fat cells. No cell number differences were observed between the groups. After eight weeks of training, the cells had been reduced to 75μ, a size which was not significantly different from the mean diameter of 72μ found for the continuously trained rats. These results concur with literature reports for both animals (Hirsch and Han, 1969) and humans (Hirsch and Knittle, 1970; Bray, 1970; Brook, 1970), in which weight loss in adults was induced by various combinations of reduced food intake and increased activity. Since cell number is not reducible by these methods, permanent weight loss could not be expected to occur unless the activity and dietary restrictions were maintained. These results agree with the conclusions of Goss (1964, 1970) regarding the irreversible nature of a cellular commitment in non-mitotic tissues, and further justify the classification of adipose tissue as such a static tissue.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary

The effects of endurance training, detraining and late training (weight loss), upon the size and number of epididymal fat cells were investigated in 63 male rats. Two successive eight week growth periods, from seven to sixteen weeks and from 16 to 24 weeks respectively, were studied. Fifteen weeks was considered to be a critical age beyond which the number of mature fat cells is no longer subject to change. Body weight, fat pad weights, Fatness Index and pad lipid content were determined and suspensions of fixed isolated fat cells were used for microscopic measurement of cell diameter. Cell number was determined indirectly from the lipid contents of mean cell and whole pad.

Training, whether early, late or continuous, had a substantial effect upon limiting the accumulation of fat in the body. At both 16 and 24 weeks of age, the sedentary groups had significantly larger fat pads and Fatness Indices than did the training groups. Weight loss in adult rats led

to a significant reduction in body fat content, while detraining resulted in an even more significant accumulation of adipose mass.

Between seven and sixteen weeks of age, cell volume increase two-fold in trained and four-fold in sedentary rats, while cell number showed greater than a two-fold increase in both groups. Beyond 16 weeks of age, there was no change in cell number and also no change in cell size. Training, whether early, late or continuous, did not significantly affect cell number, but was effective in maintaining or reducing cell volume to a level approximately half of that found in sedentary rats. All the effects of detraining and weight loss in adult rats were evident in the cell size changes. Detraining was accompanied by a return of cell size to sedentary levels, so the effects of early training were not sustained.

Though training had no significant effects upon cell number in this study, all four groups which had received some training had larger cell complements than did the sedentary groups. Significance was not attained because of the wide variation in cell number between the rats within each group. This suggests the need for a more powerful experiment with considerably more subjects and preferably a repeated measures design.

The extent to which these results can be generalized to humans is severely limited, due to large differences in the life-span and the duration of the pre-maturation periods.

Conclusions

Within the limitations of the study, the following conclusions were drawn:

(1) fat cell number continues to increase until 16 weeks of age in the rat, but endurance training beyond very early life (after seven weeks of age) does not affect significantly the adult fat cell complement;

(2) physical training is extremely effective in limiting adipocyte hypertrophy and, by this effect alone, can reduce two-fold, the accumulation of fat which normally occurs during growth.

(3) detraining and late training (weight loss) in adult rats result in significant alterations in fat cell size, but no modification of the fat cell number.

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APPENDIX A

DATA SHEET, CALCULATION SHEETS

Name: Group:

(A) CELL SIZE DISTRIBUTION DATA

Micrometer Scale Range	Tally	f	Diameter (μ)	fD	Volume (pl.)	fV
0.25 - 0.5			11.8425		1.1595	
0.5 - 0.75			19.7375		4.5091	
0.75 - 1.0			27.6325		11.7237	
1.0 - 1.25			35.5375		24.3493	
1.25 - 1.5			43.4225		43.9332	
1.5 - 1.75			51.3175		72.0172	
1.75 - 2.0			59.2125		110.1516	
2.0 - 2.25			67.1075		159.8808	
2.25 - 2.5			75.0025		222.7510	
2.5 - 2.75			82.8975		300.3080	
2.75 - 3.0			90.7925		394.0979	
3.0 - 3.25			98.6875		505.6666	
3.25 - 3.5			106.5825		636.5602	
3.5 - 3.75			114.4775		788.3246	
3.75 - 4.0			122.3725		962.5358	
4.0 - 4.25			130.2675		1160.6498	
4.25 - 4.5			138.1625		1384.3027	
4.5 - 4.75			146.0575		1634.9950	
		N=		ΣfD =		ΣfV =

(B) PERCENT LIPID IN TISSUE

Size of heptane aliquot (mls.)	Absorbance Readings 1	2	3	4	$\bar{x}$	Lipid in aliquot (mg.)	Lipid/ml. heptane	Total lipid (mg.) in tissue sample (32 ml. heptane)	Wt. of tissue sample (mg.)	% Lipid in tissue

Date:

Name: Group: Treatment:

Age (wks) = Body Weight (g.) = Fat Pad Weights (mg.) (larger pad used) Left / Right

$\bar{x}$ =

% Lipid (in pieces of tissue summing to ~ 100 mg) =

Mean Cell Diameter $(\bar{D} \pm \sigma) = \frac{\Sigma fD}{N} \pm \sqrt{\frac{(N.\Sigma fD^2) - (\Sigma fD)^2}{N\ (N-1)}} = \pm \mu$

Mean Cell Volume $(\bar{V}) = \frac{\Sigma fV}{N} =$ pl.

Mean Weight of Lipid/Cell $(\bar{M}) = \bar{V}$ (pl.). Density of triolein $(.915\ m\mu g/pl.) \cdot 10^{-3} =$ $\mu g.$ lipid/cell

Total N° of Cells (in largest fat pad) = pad weight (mg.) $\cdot \frac{\%\ lipid}{100} \cdot 10^3 \cdot \frac{1}{\mu g.\ lipid/cell} =$

$\times 10^6$ cells/pad

SURFACE AREA (S.A.) = $3.14159 \times D^2$

MEAN SURFACE AREA = $\frac{f\ S.A.}{N}$

APPENDIX B

RAW DATA

TABLE B1 WEIGHT PROGRESS FOR GROUPS (MEANS)

Group	AGE IN WEEKS																	
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
I	201																	
S	182	229	258		285	-	357	377	388	426	447							
T	183	226	242	275	296	-	326	340	359	374	382							
SS	-	-	250	279	320	345	358	372	388	399	-	412	428	432	426	432	435	443
TT	-	-	252	290	313	334	347	365	378	386	-	396	409	421	415	436	438	437
ST	-	-	252	277	320	338	355	364	378	390	-	404	408	400	392	418	418	427
TS	-	-	252	286	312	332	340	356	369	377	-	406	412	440	451	477	488	506

TABLE B2 FREQUENCY TABLE OF CELL SIZES
GROUP I (INITIAL GROUP; 7 WEEKS OF AGE)

Rat Number / Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	4	0	11	1	1	0	0	5	0
27.63	17	4	18	12	6	5	10	6	7
35.54	14	5	9	19	10	11	16	7	12
43.42	18	14	19	31	20	19	29	30	29
51.32	32	27	40	74	30	75	54	64	80
59.21	76	85	71	110	83	84	84	48	62
67.11	82	77	50	42	74	31	51	10	19
75.00	35	27	12	11	33	8	7	4	4
82.90	8	3	1	1	3	2	2	1	1
90.79	2	0	0	0	0	2	0	0	1
98.69	0	0	0	1	0	0	0	0	0
106.58	0	0	0	0	0	0	0	0	0
114.48	0	0	0	0	0	0	0	0	0
122.37	0	0	0	0	0	0	0	0	0
130.27	0	0	0	0	0	0	0	0	0
138.16	0	0	0	0	0	0	0	0	0
146.06	0	0	0	0	0	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES

GROUP S (SEDENTARY; 16 WEEKS OF AGE)

Rat Number / Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	0	0	0	0	0	0	0	1	0
27.63	0	0	0	5	2	0	1	1	0
35.54	0	1	4	4	1	0	1	1	0
43.42	1	1	2	4	0	4	0	3	2
51.32	2	0	3	4	1	2	1	1	2
59.21	2	3	8	3	7	3	2	5	6
67.11	7	2	5	8	9	17	10	7	27
75.00	19	4	26	18	13	35	28	28	52
82.90	29	19	51	33	39	61	46	50	68
90.79	56	44	66	44	45	51	72	63	56
98.69	30	49	49	57	50	39	46	27	28
106.58	41	25	26	33	35	11	27	20	13
114.48	24	17	8	13	10	4	7	7	3
122.37	8	8	5	4	6	0	2	3	0
130.27	2	1	3	3	3	0	0	3	0
138.16	0	0	0	1	0	0	0	0	0
146.06	0	0	0	0	0	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES
GROUP T (TRAINED; 16 WEEKS OF AGE)

Rat Number / Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	0	0	0	0	0	0	0	0	0
27.63	8	2	3	1	4	0	1	1	1
35.54	7	4	1	6	3	2	2	2	5
43.42	4	1	3	12	1	2	6	12	13
51.32	7	7	13	22	14	10	17	38	50
59.21	15	23	42	67	63	30	59	83	90
67.11	40	54	75	84	82	76	79	69	76
75.00	54	90	66	66	61	101	71	42	39
82.90	50	56	33	28	23	47	21	17	9
90.79	33	35	12	7	8	16	8	9	3
98.69	12	12	2	1	2	4	1	2	0
106.58	4	5	1	1	0	1	0	0	0
114.48	1	0	0	0	0	1	0	0	0
122.37	0	0	0	0	0	0	0	0	0
130.27	0	0	0	0	0	0	0	0	0
138.16	0	0	0	0	0	0	0	0	0
146.06	0	0	0	0	0	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES
GROUP SS (SEDENTARY; 24 WEEKS OF AGE)

Rat Number / Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	0	0	0	0	0	0	0	0	0
27.63	0	0	0	0	0	1	0	0	0
35.54	0	0	1	0	0	2	2	1	1
43.42	0	2	1	1	0	1	3	2	2
51.32	1	1	3	1	1	0	2	2	2
59.21	2	3	1	4	1	2	4	4	11
67.11	9	4	5	3	2	13	8	13	30
75.00	19	14	15	5	8	30	22	24	70
82.90	30	23	29	22	22	48	39	33	64
90.79	39	37	41	28	41	60	47	38	35
98.69	25	37	45	40	40	36	33	34	21
106.58	18	28	46	41	33	14	25	23	4
114.48	23	15	18	27	16	6	16	11	1
122.37	10	2	10	14	8	0	6	10	1
130.27	2	1	4	3	2	0	4	1	0
138.16	1	2	4	1	0	0	0	0	0
146.06	1	0	2	0	1	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES
GROUP TT (TRAINED; 24 WEEKS OF AGE)

Rat Number Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	0	0	0	0	0	0	0	0	0
27.63	3	4	2	1	0	0	0	3	0
35.54	3	4	1	3	5	0	0	3	7
43.42	3	5	1	8	3	14	2	3	7
51.32	4	4	2	16	9	28	3	12	8
59.21	9	17	9	48	24	66	18	48	40
67.11	25	38	34	65	57	40	40	81	54
75.00	50	50	64	50	56	35	72	67	50
82.90	49	36	67	25	35	7	40	38	22
90.79	27	17	34	9	18	1	24	29	6
98.69	11	8	19	5	4	0	14	12	0
106.58	5	2	3	2	1	0	3	1	0
114.48	5	1	2	0	0	0	1	2	0
122.37	2	0	0	0	0	0	0	0	0
130.27	0	0	0	0	0	0	0	0	0
138.16	0	0	0	0	0	0	0	0	0
146.06	0	0	0	0	0	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES
GROUP ST (TRAINED; 16-24 WEEKS OF AGE)

Rat Number Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	0	0	0	1	1	0	0	0	1
27.63	2	2	1	4	0	0	5	2	4
35.54	1	1	0	1	2	6	1	4	3
43.42	3	0	1	3	1	2	3	5	5
51.32	6	3	2	1	3	4	6	13	29
59.21	16	8	16	11	21	33	38	33	85
67.11	45	22	47	24	65	60	82	43	86
75.00	55	36	82	55	72	60	111	43	72
82.90	57	53	68	53	59	44	54	18	30
90.79	32	41	39	45	27	15	29	10	4
98.69	11	30	23	18	12	6	13	3	1
106.58	3	9	6	9	6	1	4	1	0
114.48	2	7	2	1	0	0	0	0	0
122.37	0	2	2	0	0	0	0	0	0
130.27	0	1	0	0	1	0	0	0	0
138.16	0	0	0	0	0	0	0	0	0
146.06	0	0	0	0	0	0	0	0	0

TABLE B2 (continued)

FREQUENCY TABLE OF CELL SIZES
GROUP TS (DETRAINED; 16-24 WEEKS OF AGE)

Rat Number / Cell Diameter (μ)	1	2	3	4	5	6	7	8	9
11.84	0	0	0	0	0	0	0	0	0
19.74	4	0	0	0	2	0	0	0	0
27.63	6	0	5	2	5	0	3	2	3
35.54	6	0	2	0	4	1	2	2	0
43.42	4	1	3	3	0	0	3	1	3
51.32	2	1	2	1	3	2	0	2	4
59.21	3	0	3	1	3	8	1	1	32
67.11	5	3	10	4	8	18	8	5	56
75.00	9	5	20	9	23	27	27	15	59
82.90	15	24	38	24	39	48	54	36	37
90.79	29	38	43	41	58	43	56	51	18
98.69	30	37	46	43	46	33	51	43	5
106.58	31	41	24	49	36	10	35	38	3
114.48	28	22	9	29	14	3	8	15	1
122.37	18	15	5	7	4	1	2	8	0
130.27	8	6	0	4	3	0	1	2	0
138.16	5	0	0	0	1	0	3	0	0
146.06	5	0	0	1	0	0	0	0	0

TABLE B3 RAW DATA FOR GROUP I (INITIAL GROUP; 7 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg.)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	199	596	584	5.93	288	58.83 ±14.19	125.42	.115	74.61	3.84
2	222	642	653	5.83	242	60.97 ±10.07	129.41	.118	66.99	3.66
3	201	571	545	5.55	231	53.92 ±14.38	99.49	.091	64.37	3.94
4	217	556	574	5.21	302	54.66 ±11.20	97.31	.089	59.54	3.78
5	200	544	471	5.01	260	59.82 ±11.53	125.32	.115	73.22	3.24
6	192	460	441	4.69	237	55.72 ±10.23	101.02	.092	67.74	3.30
7	199	433	497	4.69	253	55.19 ±11.18	99.75	.091	58.31	2.97
8	186	406	427	4.48	175	51.41 ±10.90	81.53	.075	68.10	3.80
9	194	391	374	3.94	215	53.04 ±10.02	87.73	.080	62.86	3.00
$\bar{X}$	201.11	511.0	507.3	5.04	244.8	55.95	105.22	.096	66.19	3.50
σ	11.52	90.28	88.75	.66		3.24	17.35	.016	5.56	.38

1. Rats are arranged in rank order according to Fatness Index.
2. Fatness Index = mg total epididymal fat/g. body weight.
3. Assumes a constant lipid density equal to that of triolein (.915).
4. Number of cells in mean fat pad (largest pad was used for all other groups).

TABLE B3 (continued) RAW DATA FOR GROUP S (SEDENTARY; 16 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Lipid[3] Cell Vol. (pl.)	Per Cell (μg.)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	445	4634	4648	20.86	221	94.86 ±15.20	483.02	.442	81.37	8.56
2	440	4046	4045	18.39	174	96.64 ±14.02	503.50	.461	66.90	5.88
3	447	4086	3916	17.90	256	89.40 ±15.97	410.66	.376	78.97	8.59
4	491	3892	3584	15.23	234	90.18 ±19.79	437.11	.400	83.91	8.17
5	424	3276	2979	14.75	221	92.26 ±16.23	449.66	.411	91.38	7.28
6	472	3086	3295	13.52	227	85.40 ±12.96	350.04	.320	96.96	9.97
7	438	2936	2972	13.49	243	89.78 ±13.07	404.30	.370	85.60	6.88
8	449	2724	3185	13.16	220	87.96 ±15.97	392.12	.359	82.01	7.28
9	419	2366	2558	11.75	257	83.54 ±12.36	327.17	.299	88.22	7.54
$\bar{x}$	447	3450	3464.7	15.45	228.1	90.00	417.51	.382	83.92	7.79
σ	22.39	748.8	648.3	2.98		4.19	57.76	.053	8.45	1.18

TABLE B3 (continued) RAW DATA FOR GROUP T (TRAINED; 16 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg.)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	449	2728	2598	11.86	235	74.43 ±16.86	248.70	.228	77.89	9.34
2	394	2227	2282	11.44	289	75.68 ±12.98	248.25	.227	78.82	7.92
3	367	1949	1939	10.59	251	69.78 ±11.67	194.16	.178	84.22	9.24
4	392	2009	2068	10.40	295	66.46 ±11.86	169.80	.155	74.54	9.92
5	379	1782	1752	9.32	261	67.50 ±11.30	175.79	.161	87.65	9.71
6	391	1813	1770	9.16	290	72.72 ±10.71	216.13	.198	96.59	8.86
7	409	1627	1410	7.42	265	67.61 ±10.64	175.31	.160	91.37	9.27
8	358	1358	1275	7.35	275	64.32 ±11.57	154.61	.141	55.05	5.28
9	303	1172	1048	7.33	286	61.92 ±10.36	136.16	.124	68.34	6.43
$\bar{X}$	382.4	1851.6	1793.5	9.43	271.9	68.94	190.99	.175	79.38	8.44
σ	39.67	461.7	494.5	1.77		4.63	39.54	.036	12.61	1.60

TABLE B3 (continued) RAW DATA FOR GROUP SS (SEDENTARY; 24 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg.)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	422	5205	4458	22.90	180	94.52 ±16.72	486.33	.445	85.98	10.06
2	455	4665	5411	22.14	169	94.11 ±15.45	473.08	.433	83.85	10.48
3	414	4193	3753	19.19	225	97.21 ±17.13	527.57	.483	98.32	8.54
4	458	4160	4115	18.07	190	99.60 ±15.62	556.55	.509	62.41	5.10
5	425	3675	3902	17.83	175	97.56 ±13.74	517.44	.473	84.74	6.98
6	508	4131	3989	15.98	213	87.05 ±13.42	370.86	.339	67.92	8.27
7	414	3285	3196	15.65	211	91.17 ±17.15	439.86	.402	80.28	6.55
8	472	3605	3207	14.43	196	90.51 ±16.87	430.09	.394	67.88	6.22
9	421	2610	2559	12.28	242	79.99 ±12.14	288.37	.264	90.63	8.96
$\bar{x}$	443.2	3947.7	3843.3	17.61	200.1	92.41	454.46	.416	80.22	7.91
σ	32.34	762.8	822.8	3.47		6.09	84.02	.077	11.85	1.81

TABLE B3 (continued) RAW DATA FOR GROUP TT (TRAINED; 24 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg.)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	456	2866	2932	12.71	198	79.27 ±16.88	297.69	.272	74.03	7.97
2	408	2605	2553	12.64	186	73.26 ±15.25	233.09	.213	61.57	7.52
3	491	2522	2510	10.25	238	79.48 ±12.52	283.75	.260	89.32	8.68
4	456	2344	2289	10.16	232	68.33 ±12.85	136.47	.171	86.68	11.91
5	487	2542	2205	9.75	212	71.95 ±12.56	214.13	.196	80.99	10.51
6	357	1691	1737	9.60	191	62.48 ±10.09	139.21	.127	79.08	10.78
7	401	1857	1911	9.40	217	76.97 ±11.92	257.92	.236	83.34	6.75
8	394	1524	1468	7.59	299	72.04 ±13.70	218.69	.200	80.72	6.15
9	488	1838	1748	7.35	194	67.39 ±11.90	176.30	.161	72.15	8.22
$\bar{x}$	437.6	2198.8	2150.3	9.94	218.6	72.35	223.03	.204	78.65	8.72
σ	48.89	476.1	471.7	1.86		5.69	51.51	.047	8.41	1.95

TABLE B3 (continued) RAW DATA FOR GROUP ST (TRAINED; 16-24 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	432	3849	3072	16.02	233	76.76 ±13.50	260.08	.238	87.99	14.23
2	451	3535	3252	15.05	215	84.22 ±15.38	345.39	.316	87.13	9.74
3	439	3028	2734	13.13	289	79.54 ±12.61	285.41	.261	86.99	10.09
4	453	2936	2784	12.63	226	79.93 ±15.06	295.80	.271	82.53	8.95
5	451	2792	2591	11.98	270	76.26 ±12.77	253.57	.232	87.95	10.58
6	432	2083	2286	10.11	231	72.27 ±12.28	216.06	.198	66.23	7.66
7	374	1783	1550	8.70	346	73.72 ±12.63	229.58	.210	83.47	7.08
8	429	1784	1648	8.00	175	68.28 ±13.62	187.80	.172	74.56	7.74
9	383	1550	1333	7.71	320	65.92 ±11.41	168.61	.151	81.38	8.37
$\bar{x}$	427.1	2593.3	2361.1	11.48	256.1	75.21	248.70	.228	82.02	9.38
σ	20.09	826.9	698.1	3.03		5.82	56.21	.051	7.33	2.17

TABLE B3 (continued) RAW DATA FOR GROUP TS (DETRAINED; 16-24 WEEKS OF AGE)

Rat[1] Number	Body Wt. (g.)	Left Pad Wt. (mg.)	Right Pad Wt. (mg.)	Fatness[2] Index (mg/g.)	No. of Cells Sized	Mean Cell Diam. ($\mu \pm \sigma$)	Mean Cell Vol. (pl.)	Lipid[3] Per Cell (μg)	Pad Lipid Content (% Wt.)	Cell[4] Number ($\times 10^6$)
1	535	5556	6160	21.90	208	95.84 ±27.84	568.78	.520	98.31	11.64
2	517	5547	5547	21.46	193	99.83 ±14.72	556.72	.509	95.41	10.38
3	513	4580	4952	18.58	210	88.50 ±18.25	407.48	.373	91.19	12.11
4	485	4232	3818	16.60	218	97.52 ±16.74	528.25	.483	78.08	6.84
5	537	4454	4150	16.02	249	90.00 ±19.37	432.18	.395	67.36	7.59
6	540	4479	3663	15.08	194	85.14 ±13.46	348.88	.319	91.10	12.78
7	475	3059	3120	13.01	254	90.30 ±16.27	423.30	.387	98.11	7.90
8	500	3339	3051	12.78	221	93.47 ±16.42	466.97	.427	95.73	7.48
9	451	1068	1005	9.03	221	72.97 ±12.91	224.01	.205	79.65	8.04
$\bar{x}$	505.9	4146.0	4051.8	16.05	218.7	90.40	439.62	.402	88.33	9.42
σ	30.80	1145.8	1311.7	4.19		8.02	109.01	.100	10.82	2.30

TABLE B4 AVERAGE SIZE DISTRIBUTIONS (FREQUENCIES EXPRESSED AS A PER CENT OF THE TOTAL NUMBER OF CELLS COUNTED)

Group Diameter	I	S	T	SS	TT	ST	TS
11.84	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19.74	1.00	0.05	0.00	0.00	0.00	0.13	0.30
27.63	3.86	0.44	0.86	0.06	0.66	0.87	1.32
35.54	4.68	0.58	1.31	0.39	1.32	0.82	0.86
43.42	9.49	0.83	2.21	0.67	2.34	1.00	0.91
51.32	21.61	0.78	7.27	0.72	4.37	2.91	0.86
59.21	31.91	1.90	19.29	1.78	14.18	11.32	2.64
67.11	19.79	4.48	25.95	4.83	22.06	20.56	5.94
75.00	6.40	10.86	24.11	11.49	25.11	25.42	9.86
82.90	1.00	19.29	11.61	17.21	16.22	18.92	16.01
90.79	0.23	24.21	5.35	20.32	8.39	10.50	19.16
98.69	0.05	18.27	1.47	17.27	3.71	5.08	16.97
106.58	0.00	11.25	0.49	12.88	0.86	1.69	13.57
114.48	0.00	4.53	0.08	7.38	0.56	0.52	6.55
122.37	0.00	1.75	0.00	3.39	0.10	0.17	3.04
130.27	0.00	0.73	0.00	0.94	0.10	0.09	1.22
138.16	0.00	0.05	0.00	0.44	0.00	0.00	0.46
146.06	0.00	0.00	0.00	0.22	0.00	0.00	0.30
$\bar{x}$ (μ)	56.19	89.72	68.86	91.98	72.49	75.00	90.29
σ (μ)	±12.05	±15.67	±12.81	±16.52	±14.17	±14.20	±19.31

APPENDIX C

STATISTICAL PROCEDURES

Analysis of Variance

A randomized group design analysis of variance (Edwards, 1968: 118-120) with seven treatments (k) and nine subjects (n) in each treatment was used to analyse the treatment effect on each of the seven variables.

Basic summary table:

Source of Variation	Sum of Squares	df	Mean Square	F
Treatments		k-1		
Within treatments (error)	_____	k(n-1)		
Total		(n-1)		

where: Total sum of squares $= \sum_{1}^{kn} X_{kn}^{2} - \frac{(\Sigma X..)^2}{kn}$

Treatment sum of squares $= \sum_{1}^{k} \frac{(\Sigma X_{k.})^2}{n} - \frac{(\Sigma X..)^2}{kn}$

Within treatment sum of squares = total S.S. - treatment S.S.

Mean square for treatments = treatments S.S. $\div$ (k-1)

Mean square within treatments = within treatments S.S. $\div$ k(n-1)

$F = M.S._T \div M.S._W$

Newman-Keuls Multiple Comparison Procedure

The Newman-Keuls multiple comparison test is a modified studentized range statistic (q) used to probe the significance of the differences between ordered means (Winer, 1962: 80-87). Thus, for any two means in an array,

$$q = \frac{\bar{X}_1 - \bar{X}_2}{S.E._{\bar{X}}} \quad \text{and} \quad \bar{X}_1 - \bar{X}_2 = q \cdot S.E._{\bar{X}}.$$

The critical value for this comparison is $q_{1-\alpha}$ (r, d.f.) and the shortest significant range for the mean difference is $q_{1-\alpha}$ (r, d.f.) $\cdot$ $S.E._{\bar{X}}$.

In each case, r is the number of steps that the two means are apart on an ordered scale and d.f. is the number of degrees of freedom associated with the error estimate.

If variances are assumed to be homogeneous, a pooled estimate of the standard error can be used. Thus,

$$S.E._{\bar{X}} = \sqrt{\frac{\text{MS error}}{n}}$$

where n is the number of observations making up each mean.

If $q > q_{1-\alpha}$(r, d.f.) or if $\bar{X}_1 - \bar{X}_2 > q_{1-\alpha}$ (r, d.f.) $\cdot$ $\sqrt{\frac{\text{MS error}}{n}}$, then the difference between the two means is significant at the level of .

APPENDIX D

RESULTS: ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON SUMMARY TABLES

TABLE D1 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR BODY WEIGHT

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	513336.4	6	85556.1	80.5	< 0.001
Within	59514.2	56	1062.8		
Total	572850.6	62			

Group Mean	I 201.1	T 382.4	ST 427.1	TT 435.6	SS 443.2	S 447.2	TS 505.9	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
I 201.1		181.3	226.0	236.5	242.1	246.1	304.8	R_2=30.87	40.98
T 382.4			44.7	55.2	60.8	64.8	123.5	R_3=37.07	46.74
ST427.1				10.5	16.1	20.1	78.8	R_4=40.76	50.22
TT435.6					5.6	9.6	68.3	R_5=43.48	52.72
SS433.2						4.0	62.7	R_6=45.44	54.57
S 447.2							58.7	R_7=47.07	56.09

Group α=0.05	I	T	ST	TT	SS	S	TS
Group α=0.01	I	T	ST	TT	SS	S	TS

TABLE D2 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR LARGEST PAD WEIGHT

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	96,024,345.6	6	16,004,057.6	28.6	< 0.001
Within	31,288,684.4	56	558,726.5		
Total	127,313,030.0	62			

Group Mean	I 523.7	T 1864.3	TT 2217.2	ST 2615.9	S 3550.9	SS 4055.8	TS 4261.2	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
I 523.7		1340.6	1693.5	2092.2	3027.2	3532.1	3737.5	R_2=705.7	936.8
T 1864.3			352.9	751.6	1686.6	2191.5	2396.9	R_3=847.4	1068.6
TT2217.2				398.7	1333.7	1838.6	2044.0	R_4=931.9	1148.1
ST2615.9					93.5	1439.9	1645.3	R_5=994.0	1205.2
S 3550.9						504.9	710.3	R_6=1038.7	1247.5
SS4055.8							205.4	R_7=1076.0	1282.3

Group α=0.05	I	T	TT	ST	S	SS	TS

Group α=0.01	I	T	TT	ST	S	SS	TS

TABLE D3 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR FATNESS INDEX

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	1072.2	6	178.7	22.9	<0.001
Within	437.4	56	7.8		
Total	1509.6	62			

Group	I	T	TT	ST	S	SS	TS	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
Mean	5.04	9.43	9.94	11.48	15.45	16.05	17.61		
I 5.04		4.39	4.90	6.44	10.41	11.01	12.57	R_2=2.64	3.51
T 9.43			0.51	2.05	6.02	6.62	8.18	R_3=3.17	4.00
TT9.94				1.54	5.51	6.11	7.67	R_4=3.49	4.30
ST11.48					3.97	4.57	6.13	R_5=3.72	4.51
S 15.45						0.06	2.16	R_6=3.89	4.66
TS16.05							1.56	R_7=4.03	4.80

Group α=0.05	I	T	TT	ST	S	TS	SS

Group α=0.01	I	T	TT	ST	S	TS	SS

TABLE D4 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR MEAN CELL DIAMETER

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	10021.0	6	1670.2	53.8	<0.001
Within	1736.7	56	31.0		
Total	11757.7	62			

Group	I	T	TT	ST	S	TS	SS	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
Mean	55.95	68.94	72.35	75.21	90.00	90.39	92.41		
I 55.95		12.99	16.4	19.26	34.05	34.44	36.46	R_2=5.28	7.01
T 68.94			3.41	6.27	21.06	21.45	23.47	R_3=6.34	8.00
TT72.35				2.86	17.65	18.04	20.06	R_4=6.98	8.59
ST75.21					14.79	15.18	17.20	R_5=7.44	9.02
S 90.00						0.39	2.41	R_6=7.77	9.34
TS90.39							2.02	R_7=8.05	9.60

Group α=0.05	I	T	TT	ST	S	TS	SS

Group α=0.01	I	T	TT	ST	S	TS	SS

TABLE D5 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR MICROGRAMS OF LIPID PER CELL

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	0.870	6	0.145	40.48	<0.001
Within	0.201	56	0.004		
Total	1.071	62			

Group Mean	I 0.096	T 0.175	TT 0.204	ST 0.228	S 0.382	TS 0.402	SS 0.416	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
I 0.096		0.079	0.108	0.132	0.286	0.306	0.320	R_2=0.057	0.075
T 0.175			0.029	0.053	0.207	0.227	0.241	R_3=0.068	0.086
TT0.204				0.024	0.178	0.198	0.212	R_4=0.075	0.092
ST0.228					0.154	0.174	0.188	R_5=0.080	0.097
S 0.382						0.020	0.034	R_6=0.084	0.100
TS0.402							0.014	R_7=0.087	0.103

Group α=0.05	I	T	TT	ST	S	TS	SS

Group α=0.01	I	T	TT	ST	S	TS	SS

TABLE D6 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR PAD LIPID CONTENT

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	2533.2	6	422.2	4.60	<0.001
Within	5145.2	56	91.9		
Total	7678.4	62			

Group Mean	I	TT	T	SS	ST	S	TS	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
	66.19	78.65	79.38	80.22	82.02	83.92	88.33		
I 66.19		12.46	13.19	14.03	15.83	17.73	22.14	R_2= 9.06	12.03
TT 78.65			0.73	1.57	3.37	5.27	9.68	R_3=10.88	13.72
T 79.38				0.84	2.64	4.54	8.95	R_4=11.96	14.68
SS 80.22					1.80	3.70	8.11	R_5=12.76	15.47
ST 82.02						1.90	6.31	R_6=13.33	16.01
S 83.93							4.41	R_7=13.81	16.46

Group α=0.05	I	TT	T	SS	ST	S	TS

Group α=0.01	I	TT	T	SS	ST	S	TS

TABLE D7 ANALYSIS OF VARIANCE AND MULTIPLE COMPARISON FOR CELL NUMBER ($x10^6$)

Source of Variation	S.S.	d.f.	M.S.	F	P
Treatment	223,285,185.1	6	37,214,197.5	12.3	<0.001
Within	169,612,122.8	56	3,028,787.9		
Total	392,897,307.9	62			

Group Mean	I 3.50	S 7.79	SS 7.91	T 8.44	TT 8.72	ST 9.38	TS 9.42	Shortest Significant Ranges α=0.05	Shortest Significant Ranges α=0.01
I 3.50		4.29	4.41	4.94	5.22	5.88	5.92	R_2=1.65	2.19
S 7.79			0.12	0.65	0.93	1.59	1.63	R_3=1.98	2.49
SS7.91				0.53	0.81	1.47	1.51	R_4=2.18	2.68
T 8.44					0.28	0.94	0.97	R_5=2.32	2.81
TT8.72						0.66	0.69	R_6=2.42	2.91
ST9.38							0.03	R_7=2.51	2.99

Group α=0.05	I	S	SS	T	TT	ST	TS

Group α=0.01	I	S	SS	T	TT	ST	TS